MECHANISMS FOR REDUCING PHOTORESPIRATION IN SUBMERSED AQUATIC ANGIOSPERMS

BY

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To my wife (spouse),

Cindy

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KEY TO ABBREVIATIONS

CAM	Crassulacean Acid Metabolism
DNPH	2,4-dinitrophenylhydrazine
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetate
EU	Enzyme Units
Hepes	[N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate]
IgG	gamma immunoglobulin
INH	isonicotinic acid hydrazide
IU	International Unit
L/D	Light/Dark
Mes	2-[N-morpholino] ethanesulfonic acid
PCO	Photorespiratory Carbon Oxidation
PCR	Photosynthetic Carbon Reduction
PEP	phosphoenolpyruvate
3-PGA	3-phosphoglycerate
PR	photorespiration
PVP	polyvinyl pyrrolidone
RuBP	ribulose-1,5-bisphosphate
RuBPCO	ribulose-1,5-bisphosphate-carboxylase-oxygenase
SP	sugar monophosphate
SP_2	sugar bisphosphate
TPP^+	tetraphenyl phosphonium
Tris	tris(hydroxymethyl) aminomethane
Γ	CO_2 compensation point

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MECHANISMS FOR REDUCING PHOTORESPIRATION IN SUBMERSED AQUATIC ANGIOSPERMS

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The biochemical mechanisms regulating photorespiration in submersed angiosperms were examined in Myriophyllum spicatum L. and Hydrilla verticillata (L.F.) Royal. Oxygen inhibition of net photosynthesis at 330 µl CO_2/l was 23 and 24%, respectively, and responded competitively to CO_2 in the high photorespiration state, but was 15 and 5%, respectively, and independent of the CO_2 concentration in the low photorespiration state. Carbonic anhydrase activity increased by three-fold with the induction of the low photorespiration state, but no change in the kinetic characteristics of ribulose-1,5-bisphosphate carboxylase-oxygenase occurred.

In pulse-chase experiments, C_4 acids accounted for greater than 50% of the initial products in Hydrilla, but label in malate decreased during the chase only in the low photorespiration state. Carbon assimilation was almost exclusively via the Calvin cycle in Myriophyllum.

Ethoxyzolamide repressed the low photorespiration state in Myriophyllum and Hydrilla as it reduced photosynthesis by 40%, and increased O_2

inhibition, the apparent Km(CO_2) of photosynthesis, the CO_2 compensation point and, for Myriophyllum, the fraction of label fixed into photorespiratory intermediates. Net photosynthesis in high photorespiration Myriophyllum and Hydrilla plants was inhibited only slightly by ethoxyzolamide, and their CO_2 compensation points, and those of the terrestrial plants, Sorghum, Moricandia and Nicotiana, were unaffected.

Treatment with isonicotinic acid hydrazide caused label to accumulate in glycine, which then represented 25 and 47% of the labeled products in low and high photorespiration Myriophyllum, respectively, and 9 and 14% in Hydrilla, respectively. Glycine labeling and O_2 inhibition of photosynthesis appeared to be uncoupled from the external $[O_2]/[CO_2]$ ratio in low photorespiration Myriophyllum as both were affected by changes in the O_2 but not in the CO_2 concentration. Oxygen inhibition in the high photorespiration state responded to changes in both CO_2 and O_2.

It is suggested that Myriophyllum and Hydrilla exemplify two different photosynthetic mechanisms but both represent strategies, though different, for concentrating inorganic carbon. Common to both is a requirement for carbonic anhydrase, possibly to enhance the usage of accumulated CO_2 for reducing photorespiration. These data suggest that submersed angiosperms cannot be categorized in the presently recognized photosynthetic groupings.

CHAPTER I
PHOTOSYNTHETIC CARBON ASSIMILATION AND PHOTORESPIRATION IN SUBMERSED AQUATIC MACROPHYTES: A REVIEW

The pathway for carbon assimilation elucidated by Calvin, Benson, Bassham and their co-workers is central to the incorporation of carbon into the biosphere by photoautotrophic organisms (cf. Bassham and Calvin, 1957; Hatch and Boardman, 1981). Among photosynthetic organisms, atmospheric or dissolved CO_2 may be assimilated directly into the Photosynthetic Carbon Reduction cycle (PCR or Calvin cycle) by the initial carboxylation reaction or indirectly, following the release of CO_2 by an internal decarboxylation reaction. In the former case, the initial event in the fixation of CO_2 is the carboxylation of CO_2 with ribulose-1,5-bisphosphate (RuBP), catalyzed by RuBP carboxylase, while in the latter case, this carboxylation step is proceeded by an initial β-carboxylation reaction catalyzed by phosphoenolpyruvate (PEP) carboxylase. In terrestrial plants, the route of CO_2 assimilation can ultimately determine what percent of the carbon fixed flows through the Photorespiratory Carbon Oxidation cycle (PCO or photorespiratory cycle). The PCO cycle is important energetically for a plant, and it has major implications in regard to growth, productivity, and even survival in a particular habitat (Björkman, 1976; Osmond, 1981).

Higher terrestrial plant species can be divided into 4 major groups (C_3, C_4, C_3-C_4 intermediate, and Crassulacean Acid Metabolism (CAM)) depending upon their mode of photosynthetic carbon assimilation (Chollet and

Ogren, 1975; Rathnam and Chollet, 1980; Osmond and Holtum, 1981). The biochemical, physiological and ecological aspects of photosynthetic carbon metabolism, in each of these groups, have been described in detail, and several excellent review articles are available (Chollet and Ogren, 1975; Ehleringer, 1979; Rathnam and Chollet, 1980; Hatch and Boardman, 1981; Lorimer, 1981; O'Leary, 1981; Osmond, 1981; Tolbert, 1981; Somerville and Ogren, 1982). In contrast to terrestrial plants, a relatively limited amount of attention has been directed toward the study of photosynthetic carbon metabolism in submersed aquatic macrophytes and no reviews on the subject have been conducted to date. A casual perusal of the literature, however, will reveal that, while there is no consensus view on the subject, there is unanimity among investigators that submersed aquatic macrophytes exhibit some physiological and/or biochemical features which are not typical of any one of the recognized photosynthetic groups. It is the purpose of this paper to review the literature on photosynthetic and photorespiratory carbon metabolism in submersed aquatic macrophytes from a mechanistic/biochemical perspective, underlining the fact that the biochemical reactions of photosynthesis serve as a common basis from which to compare the physiological responses of photosynthesis and photorespiration in these organisms, with the same physiological responses in terrestrial higher plants.

General Features of Submersed Aquatic Macrophytes

Submersed aquatic macrophytes are plants which are adapted physiologically and morphologically to grow and photosynthesize under water. Included for discussion in this review will be species from charophytic

algae through submersed angiosperms, both freshwater and marine; but with more emphasis on the freshwater species for which there is more information available. Emergent plants such as Typha (cattail) and Spartina (cordgrass), which photosynthesize primarily in the aerial environment, will not be considered. Also excluded from discussion will be the aerial or floating-leaf forms of heterophyllic aquatic macrophyte species such as Myriophyllum brasiliense (parrot's feather) and Potamogeton amplifolius (pondweed). These species are similar to emergent species in that they exhibit few of the more unusual features characteristic of photosynthetic carbon metabolism in submersed aquatic macrophytes (Hutchinson, 1975; Lloyd et al., 1977; Salvucci and Bowes, 1982).

In general, submersed macrophytes are among the least productive of plants and exhibit correspondingly low photosynthetic capacities (Cooper, 1975). Some seagrass species, however, are regarded as highly productive (Williams and McRoy, 1982), although this is inconsistent with their relatively low photosynthetic capacity (Beer et al., 1977). In spite of their low productivity, freshwater macrophytes can rapidly form heavy stands of vegetation in an invaded area and some species have become major weed problems (cf. Van et al., 1978). The apparent prolific growth is due, in part, to a lower requirement for structural biomass components in the aquatic environment which, as evidenced by the low dry weight/fresh weight ratio, provides for volume increases without substantial dry matter gain (Westlake, 1975; Bowes et al., 1979). Low chlorophyll and soluble protein content, expressed on an area or fresh weight basis, are also characteristics of freshwater submersed macrophytes due to their high (up to 95%) water content (De Groote and Kennedy, 1977; Holaday et al., 1983). This consideration should be realized when comparing photosynthetic and

photorespiratory activity in submersed macrophytes to that in terrestrial plant species.

Physiological Aspects

Photosynthesis and the Apparent Km(CO_2)

In spite of the use of a variety of methods for measurement, published rates of net photosynthesis for a large range of freshwater macrophytes are remarkably consistent. At air-levels of CO_2 (330 µl CO_2/l, gas phase or 10 µM free CO_2, aqueous), the light saturated rate of net photosynthesis under ambient O_2 concentrations is typically 1 to 20 µmol/mg Chl·h whether measured by O_2 evolution (Van et al., 1976; Beer and Wetzel, 1981; Raven et al., 1982), ^{14}C incorporation (Brown et al., 1974; McCracken et al., 1975; Best and Meulemans, 1979; Holaday and Bowes, 1980) or infrared gas analysis (Van et al., 1976; Lloyd et al., 1977; Browse et al., 1979b; Keeley and Bowes, 1982; Raven et al., 1982; Salvucci and Bowes, 1982). Under saturating inorganic carbon concentrations, net photosynthetic rates of approximately 50-60 µmol/mg Chl·h are typically measured in O_2 electrode systems (Van et al., 1976; Beer and Wetzel, 1981); however, somewhat higher rates of approximately 150 µmol/mg Chl·h have been measured with infrared gas analysis systems (Browse et al., 1979b; Salvucci and Bowes, 1982).

Under saturating concentrations of inorganic carbon, rates of net photosynthesis reported for marine angiosperms (seagrasses) are, in general, very similar to those for freshwater macrophytes (Black et al., 1976; Downton et al., 1976; Beer et al., 1977). In a few species of seagrasses from tropical waters, carbon uptake has been reported to be substantially higher than in freshwater macrophytes (Williams and McRoy, 1982); however,

these rates are still considerably lower than the rates of most terrestrial C_3 plants (Cooper, 1975).

The concentration of CO_2 required to saturate photosynthesis in submersed aquatic macrophytes is of the order of 350-600 μM or approximately 10-20 times greater than required for terrestrial C_3 plants (Steeman Neilson, 1947; Raven, 1970; Hutchinson, 1975; Van et al., 1976; Beer et al., 1977; Browse et al., 1979b). This high requirement for CO_2 has long been recognized as a feature characteristic of photosynthesis in submersed macrophytes (Steeman Neilson, 1947; Raven, 1970). The apparent $Km(CO_2)$ values for RuBP carboxylase-oxygenase from several submersed macrophyte species are somewhat higher than in terrestrial C_3 plants (Yeoh et al., 1981), although similar to the values of the enzymes from C_4 plants and green algae (Jordan and Ogren, 1981b; Yeoh et al., 1981). Although higher, the difference in the $Km(CO_2)$ values between aquatic plants and terrestrial C_3 plants is less than two-fold, and thus insufficient to account for the very high apparent $Km(CO_2)$ values for photosynthesis typically reported in submersed macrophytes (Van et al., 1976; Browse et al., 1979b; Black et al., 1981).

The high apparent $Km(CO_2)$ for photosynthesis in submersed macrophytes has been evaluated in terms of the component resistances to CO_2 transfer and is attributed most frequently to a slow rate of CO_2 diffusion through the aqueous environment (Steeman Neilson, 1947; Raven, 1970). It is now well-established that, under unstirred conditions, the major resistance to CO_2 assimilation is diffusion through the boundary or "unstirred" layer, which can be 100-500 μm in distance (Steeman Neilson, 1947; Raven, 1970; Browse et al., 1979b; Smith and Walker, 1980; Black et al., 1981). Under well-stirred conditions, both the apparent $Km(CO_2)$ for photosynthesis and

the boundary layer decrease (Browse et al., 1979b); however, the residual resistance is still considerable as compared with photosynthesis in terrestrial plants (Van et al., 1976; Ascencio, 1979; Browse et al., 1979b; Smith and Walker, 1980; Black et al., 1981).

The bulk of the remaining resistance under nonphotorespiratory conditions has been attributed to diffusion within the boundary layer which is thought to persist as the major limitation to CO_2 assimilation even under turbulent or well-stirred conditions (Browse et al., 1979b; Black et al., 1981). Evidence for this is available in a study of photosynthesis in the amphibious moss Polytrichum formosum (Gessner, 1955), for which the apparent $Km(CO_2)$ for photosynthesis was reduced by measurement in air. However, in a more recent study, the high CO_2 requirement for net photosynthesis in four submersed angiosperm species could not be overcome by measurement in a water-saturated aerial environment (Lloyd et al., 1977). These results are confirmed by similar, but independent studies (Ascencio, 1979; Salvucci and Bowes, 1982) which report high apparent $Km(CO_2)$ values (122 and 70 μM) for net photosynthesis measured in air for Myriophyllum (at 21% O_2) and Hydrilla (at 1% O_2), respectively. These high values indicate that, under conditions which minimize the boundary layer resistance, the internal resistance to CO_2 assimilation is still considerably greater in submersed macrophytes than in terrestrial or amphibious species (Lloyd et al., 1977; Salvucci and Bowes, 1982). Components of this high resistance include carboxylation resistance, photorespiration, and dark respiration (Ascencio, 1979), indicating that these physiological processes offer substantially more resistance to CO_2 assimilation in submersed macrophytes than in terrestrial plants (Jarvis, 1971). Under so-called natural conditions, however, boundary layer

resistance can be extremely high and is probably the most important factor limiting CO_2 assimilation (Raven, 1970; Hutchinson, 1975; Browse et al., 1979b; Smith and Walker, 1980).

Bicarbonate Usage

The ability of submersed macrophytes to utilize HCO_3^- as a source of inorganic carbon for photosynthesis is widely recognized and well-documented (cf. Steeman Neilson, 1947; Raven, 1970). It should be emphasized, however, that the species of carbon that is directly incorporated during photosynthesis is determined by the carboxylase enzymes, free CO_2 for RuBP carboxylase (Cooper et al., 1969) and HCO_3^- for PEP carboxylase (Cooper and Wood, 1971), regardless of the species of carbon that is taken up by the plant. Furthermore, it is the pH at the active site of carboxylation that dictates the relative abundance of free CO_2 and HCO_3^- for use in photosynthesis, and equilibrium concentrations of these two species may be maintained, in accord with the pH, by the enzyme carbonic anhydrase (Maren, 1967). Carbonic anhydrase activity has been reported in virtually all submersed macrophytes that have been examined (Graham and Smillie, 1976; Van et al., 1976; Weaver and Wetzel, 1980).

The utilization of HCO_3^- as an exogenous source of inorganic carbon has been discussed extensively (cf. Raven, 1970; Smith and Walker, 1980) and it is generally accepted that most submersed macrophyte species utilize HCO_3^- for photosynthesis (Steeman Neilson, 1947; Van et al., 1976; Beer et al., 1977; Browse et al., 1979b; Bain and Proctor, 1980; Lucas, 1980; Prins et al., 1982a). Distinctions in the past of plants as "users" and "nonusers" of HCO_3^- may in reality be one of degree rather than an absolute (Allen and Spence, 1981). It has been suggested that

the ability to utilize HCO_3^- may be a determining factor in the distribution of freshwater macrophytes in waters of different hardness and pH (Hutchinson, 1975; Bain and Proctor, 1980), though the evidence is not conclusive.

Under natural conditions, HCO_3^- may be the predominant form of carbon available in the environment (for example in seawater) and likely supplies the bulk of carbon assimilated in photosynthesis (Raven, 1970; Van et al., 1976; Beer et al., 1977). However, when HCO_3^- and free CO_2 are present in equal molar concentrations, free CO_2 appears to be the preferred species of carbon utilized (Steeman Neilson, 1947; Raven, 1970; Van et al., 1976; Browse et al., 1979b;Allen and Spence, 1981) even for marine macrophytes (Beer et al., 1977; Benedict et al., 1980). Thus, the relative importance of HCO_3^- as a source of carbon for photosynthesis in submersed macrophytes is perhaps more dependent upon the environmental factors which determine the availability of CO_2 versus HCO_3^- in the aquatic environment, than upon the efficiency of the plant to take up HCO_3^-.

Bicarbonate utilization by submersed macrophytes is generally considered to be an active process (Steeman Neilson, 1947; Raven, 1970; Prins et al., 1982b), possibly electrogenic (Lucas et al., 1977), and exhibits polarity in some species, in that OH^- ions are extruded from the adaxial side of the leaf and the inorganic carbon species enters via the abaxial surface, while in others it is nonpolar (Steeman Neilson, 1947; Lucas, 1980; Prins et al., 1982a, 1982b). Possible mechanisms for HCO_3^- uptake or utilization have been discussed in detail (Lucas, 1980; Walker, 1980; Prins et al., 1982b) based primarily on studies with a few selected species (i.e., Chara and Potamogeton). A fundamental difference between the proposed mechanisms concerns the question of which species of inorganic

carbon actually crosses the plasmalemma. One hypothesis is that HCO_3^- can be taken up directly in a uniport or synport (H^+/HCO_3^-) mechanism (Lucas, 1980; Lucas et al., 1982). There is evidence,however, based on pH changes at the leaf surface, to support an alternative hypothesis; that is that HCO_3^- utilization results from the conversion of HCO_3^- to CO_2 near or in the cell wall due to active H^+ extrusion. This results in CO_2 uptake by passive diffusion into the cell (Ferrier, 1980; Walker et al., 1980; Prins et al., 1982b). As is discussed below, in some macrophyte species, the utilization of HCO_3^- may require certain biochemical modifications in photosynthetic carbon metabolism and further study of this relationship may help resolve some of the mechanistic details of HCO_3^- uptake/utilization.

Photorespiration

While the absolute rate of photorespiration is difficult to quantify, several methods have been used with terrestrial plants to provide estimates of the extent of photorespiratory activity (Chollet and Ogren, 1975). As most of these methods involve infrared gas analysis measurements, some additional limitations are imposed when applying these methods to plants in an aqueous environment (cf. Salvucci and Bowes, 1982; Holaday et al., in press). Estimates of photorespiration in submersed macrophytes are also complicated by the relatively high rate of dark respiration (as compared with net photosynthesis) in these plants (Lloyd et al., 1977; Bowes et al., 1978; Søndergaard and Wetzel, 1981; Salvucci and Bowes, 1982). As in terrestrial plants, dark respiration in submersed macrophytes is saturated by 1-2% O_2, gas phase (Lloyd et al., 1977; Salvucci and Bowes, 1982), and the contribution of this respiratory process can be accounted

for in any measure of photorespiration by determining whether the measured response is O_2 sensitive (Chollet and Ogren, 1975).

CO_2 evolution into CO_2-free air (water) in the light. The rate of CO_2 evolution into CO_2-free air is a useful estimate of photorespiratory activity in terrestrial plants when the rate is evaluated with respect to the net photosynthetic rate (Chollet and Ogren, 1975; Lloyd and Canvin, 1977; Holaday et al., 1982). Unlike for most terrestrial species, the rate of CO_2 evolution in the light in submersed macrophytes includes a relatively large 1% O_2 component, which should be accounted for when making comparative estimates of photorespiratory activity based on this method (Lloyd et al., 1977; Bowes et al., 1978; Salvucci and Bowes, 1982). In the few studies in which the rate of CO_2 evolution has been measured, CO_2 evolution in the light, corrected for the rate at 1% O_2, was 5 and 17% of the net photosynthetic rate at 350 µl CO_2/l for Myriophyllum and Potamogeton, respectively, in one study (Lloyd et al., 1977) and was less than 2% and greater than 15% for Myriophyllum in another (Salvucci and Bowes, 1982). The high rate of CO_2 evolution measured for Myriophyllum and Potamogeton in comparison to net photosynthesis conflict with the widely published notion that lacunal air spaces in submersed macrophytes contribute to an enhanced internal refixation (Hough and Wetzel, 1978; Søndergaard, 1979; Søndergaard and Wetzel, 1981). Instead, these rates were comparable to those obtained for terrestrial C_3 plants (cf. Lloyd and Canvin, 1977; Holaday et al., 1982), indicating that photorespiratory activity in submersed macrophytes can be similar in magnitude to that in C_3 plants. However, for some plants, the low rates that can be obtained approximate the level measured in C_3-C_4 intermediate or even C_4 plants (Morgan and Brown, 1979; Holaday et al., 1982), species with reduced

photorespiration. It has been postulated that refixation of photorespired CO_2, by accumulating in the lacunae, would account for these low rates (Søndergaard and Wetzel, 1981). However, not all submersed plants with low rates of photorespiratory CO_2 evolution have extensive lacunae and even those with a well developed lacunal system do not always show any differences in the rate when the lacunal effect is removed by splitting the leaves (Søndergaard and Wetzel, 1981; Keeley and Bowes, 1982).

Oxygen inhibition of photosynthesis. Photorespiration, unlike dark respiration, is stimulated by increasing O_2 concentrations through 100%, and this stimulation, along with a direct competitive effect of O_2 on CO_2 fixation (Laing et al., 1974), results in an inhibition of net photosynthesis by O_2 (Chollet and Ogren, 1975). The ability of O_2 to effect a change in net photosynthesis has been used to indicate the presence and estimate the extent of photorespiration in terrestrial plants (Forrester et al., 1966a; Chollet and Ogren, 1975).

Oxygen inhibition of net photosynthesis is the most frequently cited evidence for the occurrence of photorespiration in submersed macrophytes (Brown et al., 1974; Hough, 1974; Black et al., 1976; Downton et al., 1976; Van et al., 1976; Lloyd et al., 1977; Bowes et al., 1978; Hough and Wetzel, 1978; Søndergaard, 1979). However, inhibition of photosynthesis under high O_2 concentrations (i.e., above ambient) may be caused by effects on processes unrelated to photorespiration (cf. Chollet and Ogren, 1975; Shelp and Canvin, 1980) and so the degree of inhibition caused by ambient (versus low) O_2 is potentially the most reliable way to estimate photorespiration when measuring O_2 inhibition.

For terrestrial plants, the degree of inhibition is relatively constant within the various photosynthetic groups; approximately 30% in C_3

plants (Forrester et al., 1966a; Chollet and Ogren, 1975; Holaday et al., 1982), 21-25% in C_3-C_4 intermediates (Morgan and Brown, 1979; Rathnam and Chollet, 1980; Holaday et al., 1982), and little or no inhibition by 21% O_2 is measured in C_4 plants (Forrester et al., 1966b; Chollet and Ogren, 1975). In contrast, although some inhibition of net photosynthesis by 21% O_2 can be measured in virtually all submersed macrophytes, the degree varies considerably from 4 to 30% (Black et al., 1976; Downton et al., 1976; Van et al., 1976; Lloyd et al., 1977; Ascencio, 1979; Salvucci and Bowes, 1981; Søndergaard and Wetzel, 1981), even for the same species.

CO_2 compensation points. The CO_2 compensation point can be used reliably as a single determinant in categorizing terrestrial plants as C_3, C_4, or C_3-C_4 intermediate species (Chollet and Ogren, 1975; Rathnam and Chollet, 1980). With the appropriate precautions (cf. Holaday et al., in press), the CO_2 compensation point of submersed macrophytes can be used similarly as a valid measure of the photosynthesis/photorespiration ratio; evidenced by the correlation of CO_2 compensation point values with photosynthetic and photorespiratory estimates determined by other methods (Lloyd et al., 1977; Salvucci and Bowes, 1982). The CO_2 compensation point values reported for submersed aquatic macrophytes range from high, >150 to 60 µl CO_2/l (Lloyd et al., 1977; Ascencio, 1979; Søndergaard, 1979; Black et al., 1981; Søndergaard and Wetzel, 1981; Holaday et al., 1983), through intermediate, 50 to 30 (Van et al., 1976; Lloyd et al., 1977; Browse et al., 1979b; Salvucci and Bowes, 1981), to very low, 30-0 (Stanley and Naylor, 1972; Hough, 1974; Van et al., 1976; Bowes et al., 1978; Browse et al., 1980; Salvucci and Bowes, 1981; Raven et al., 1982), sometimes for the same species (Bowes et al., 1978; Ascencio, 1979; Salvucci and Bowes, 1982; Holaday et al., 1983). The continuous range of

possible CO_2 concentration points in submersed macrophytes span the range of values in terrestrial plants: C_4, 0-5; C_3-C_4 intermediate, 12-25; and C_3, 40-60 (Chollet and Ogren, 1975; Morgan and Brown, 1979; Rathnam and Chollet, 1980).

True versus apparent photosynthesis. A modification of the elegant system developed by Ludwig and Canvin (1971) for determining the extent of photorespiration in terrestrial plants, as the difference between true and apparent photosynthesis, has been used with the submersed angiosperm, Scirpus subterminalis (Søndergaard and Wetzel, 1981). Photorespiration, estimated by this technique, occurred at rates of 8% of the net photosynthetic rate at ambient O_2 (8 mg/l), 0-4% at 1 mg O_2/l, and 15-30% at 30 mg O_2/l (Søndergaard and Wetzel, 1981). Unfortunately, a total inorganic carbon concentration of 500 µM, pH 6.7 was used, and so a direct comparison with measurements of terrestrial plants at air-levels (10 µM) are not possible. It is uncertain whether this technique can be readily adapted for use with submersed macrophytes at low inorganic carbon concentrations, because the generally low rates of photosynthesis in these aquatic plants limit the attainment of high specific activities in the plant (cf. Lloyd et al., 1977). Furthermore, because this technique relies on rapid estimations of ^{14}C and ^{12}C uptake and evolution, boundary layer effects on CO_2 diffusion will be a major source of interference.

Light/dark (L/D) ratios of CO_2 evolution. In several studies with submersed macrophytes, the ratio of $^{12}CO_2$ or $^{14}CO_2$ evolved in the light versus the dark has been used to estimate the extent of photorespiratory activity (Hough, 1974, 1976; Hough and Wetzel, 1972, 1978; Søndergaard, 1979; Søndergaard and Wetzel, 1981). The original method was developed as a way to estimate photorespiration in terrestrial plants (Zelitch, 1968);

however, its theoretical basis has been criticized (Chollet and Ogren, 1975; Chollet, 1978) and these criticisms are valid when the method is used with submersed macrophytes (cf. Salvucci and Bowes, 1982). In addition, direct comparisons of L/D ratios between submersed macrophytes and terrestrial plants, as is often done, can be misleading even for indicating the simple presence or absence of photorespiration, since the relatively higher rate of dark respiration in submersed macrophytes is not usually considered (Salvucci and Bowes, 1982; Holaday et al., in press).

The Concept of Variable Photorespiration

It is obvious from the discussion above that, while there appears to be a consensus view that submersed macrophytes exhibit some photorespiratory activity, reported levels vary considerably even within a particular species. Thus, the classification of submersed aquatic macrophytes as a group, as either C_3, C_4, or even C_3-C_4 intermediates based upon the level of photorespiratory activity, is not possible.

In order to explain changes in the CO_2 compensation point that occur naturally or as a result of specific laboratory growth conditions, reports from this laboratory have suggested that variable photorespiration may be a characteristic feature of submersed macrophytes (Bowes et al., 1978; Holaday and Bowes, 1980). In recent investigations, the concept of variable photorespiration (in effect a continuum of possible photorespiratory states) has been documented for all 11 freshwater macrophytes examined, including a charophytic alga and an aquatic moss (Salvucci and Bowes, 1981; Holaday et al., in press).

Two freshwater angiosperms, Myriophyllum and Hydrilla, have been examined in detail with respect to the gas exchange features of their low

and high photorespiration states (Bowes et al., 1978; Salvucci and Bowes, 1981, 1982). In the low photorespiration state, the CO_2 compensation points of the plants can be as low as 10 µl CO_2/l, rates of CO_2 evolution in the light are usually less than 2% of net photosynthesis, and the degree of inhibition of net photosynthesis by 21% O_2 is low (5-15%) and insensitive to changes in the CO_2 concentration. Plants in the high photorespiration state exhibit a lower rate of net photosynthesis and a two- to five-fold higher apparent Km(CO_2) for net photosynthesis than plants in the low photorespiration state. Photorespiration in the high state is measurable and comparable to that in terrestrial C_3 plants; as indicated by CO_2 compensation points greater than 50 µl CO_2/l, rates of O_2-sensitive CO_2 evolution of 15% of the net photosynthetic rate, and a 25-30% inhibition of net photosynthesis by 21% O_2 at air-levels of CO_2.

The contrasting gas exchange characteristics of the high and the low photorespiration states in submersed angiosperms resemble differences in photorespiratory activity observed for unicellular green algae grown at high, as opposed to low, concentrations of CO_2 (cf. Berry et al., 1976; Hogetsu and Miyachi, 1979; Badger et al., 1980; Beardall and Raven, 1981). The level of CO_2 used during growth can also influence the photorespiration state in submersed macrophytes (Holaday et al., 1982). Other factors which influence the induction of the low photorespiration state are submergence (Salvucci and Bowes, 1981, 1982), temperature and photoperiod (Bowes et al., 1978; Holaday and Bowes, 1980; Holaday et al., in press). These latter two parameters vary seasonally and are the likely cause of seasonal changes in the photosynthetic/photorespiratory activities that have been documented for some freshwater macrophytes (Bowes et al., 1978; Søndergaard, 1979; Holaday and Bowes, 1980). It is unknown whether a

similar seasonality in the extent of photorespiratory activity exists for marine macrophytes such as seagrasses or, for that matter, whether marine macrophytes exhibit variable photorespiratory activity. It is generally recognized that certain freshwater habitats may subject submersed plants to conditions of low CO_2 availability and high O_2, light, temperature and pH; a situation in which a mechanism to minimize photorespiratory CO_2 loss would be most advantageous (Brown et al., 1974; Van et al., 1976; Hough and Wetzel, 1978; Bowes et al., 1979; Browse et al., 1980). Similar conditions can occur in some marine habitats, for example in coastal estuaries or salt marsh pools, and a mechanism adapted to reduce photorespiration would be advantageous for marine macrophytes photosynthesizing under these environmental conditions.

Biochemical Aspects

Biochemical characteristics of photosynthetic carbon metabolism in submersed macrophytes have been examined in only a comparatively small number of studies and, consequently, detailed information is limited. Many of the investigations that were conducted intended to determine whether submersed macrophytes were C_3 or C_4 plants (cf. Stanley and Naylor, 1972; Beer et al., 1980; Beer and Wetzel, 1982b). In retrospect, it should be realized that the concept of C_4 photosynthesis requires the integration of specific biochemical reactions with a specialized anatomical arrangement (Björkman, 1976; Hattersley et al., 1977; Perrot-Rechenmann et al., 1982), the latter of which is lacking in the two- to four-cell layered leaves of most submersed macrophytes (Sculthorpe, 1971; Doohan and Newcomb, 1976; Hough and Wetzel, 1977). From recent evidence it appears that the

physiological characteristics of photosynthesis in freshwater and marine submersed macrophytes are representative of neither the C_3 nor C_4 group, although certain biochemical features in some species may be quite analogous to the pathway of C_4 acid metabolism in C_4 plants.

Photosynthetic and Photorespiratory Enzymes

RuBP carboxylase-oxygenase. Ribulose-1,5-bisphosphate (RuBP) carboxylase-oxygenase activity has been measured in several submersed freshwater angiosperms (Van et al., 1976; De Groote and Kennedy, 1977; Salvucci and Bowes, 1981; Beer and Wetzel, 1982b; Valanne et al., 1982) and in some seagrasses (Beer et al., 1980). Enzyme activity in submersed macrophytes is low in comparison with most terrestrial plants (cf. Ku et al., 1979), although it is generally comparable to the maximum photosynthetic rates (50-150 μmol/mg Chl·h) reported for these plants (Van et al., 1976; Beer et al., 1980; Salvucci and Bowes, 1982). Van et al. (1976) have attributed the low photosynthetic rates of submersed macrophytes, in part, to these low RuBP carboxylase activities; however, the opposite interpretation, that low enzymatic capacity is the result rather than the cause of the high resistance to photosynthesis in submersed macrophytes, has also been presented (Black et al., 1981).

In the 12 species of freshwater macrophytes in which it has been determined, the $Km(CO_2)$ of RuBP carboxylase-oxygenase is higher than for the enzyme from terrestrial C_3 species, but similar to the $Km(CO_2)$ of the enzyme from C_4 plants and green algae (Yeoh et al., 1981). The substrate specificity factor and the $Km(CO_2)$ for RuBP carboxylase-oxygenase from Myriophyllum or Hydrilla were virtually identical in the low and high photorespiration state (APPENDIX A). The specificity factor, V_cK_o/V_oK_c (Jordan and Ogren, 1981a, 1981b), for these species is in the lower range

of the values reported for terrestrial plants (Jordan and Ogren, 1981b; APPENDIX A), indicating that the relative potential of RuBP carboxylase-oxygenase for carboxylation and oxygenation in these submersed angiosperms species is similar to that for the enzyme from terrestrial plants. The amount of RuBP carboxylase-oxygenase protein in the leaves of Hydrilla and Myriophyllum, estimated by rocket immunoelectrophoresis, coincides with the low specific activity (per mg Chl) in these plants (Salvucci and Bowes, 1981). On a chlorophyll or fresh weight basis, RuBP carboxylase-oxygenase protein in Hydrilla and Myriophyllum is two- to four-fold less abundant than in terrestrial C_3 plants (Ku et al., 1979; Rejda et al., 1981); the difference is mainly a function of a lower level of total soluble protein in submersed macrophytes (De Groote and Kennedy, 1977).

PEP carboxylase. The activity of PEP carboxylase in some submersed macrophyte species may be relatively high when evaluated with respect to the total carboxylase activity, RuBP + PEP carboxylase. This situation is clearly the case for some freshwater macrophytes and marine seagrasses (De Groote and Kennedy, 1977; Bowes et al., 1978; Ascencio, 1979; Beer et al., 1980; Salvucci and Bowes, 1981; Beer and Wetzel, 1982b) although in other species, PEP carboxylase activity is low when considered in relation to RuBP carboxylase activity (Van et al., 1976; Salvucci and Bowes, 1981; Valanne et al., 1982). Seasonal variations in the ratio of RuBP/PEP carboxylase activity have been reported (Bowes et al., 1978; Beer and Wetzel, 1982b; Holaday et al., 1982) and, for some species, a decrease in the RuBP/PEP carboxylase ratio can be correlated with an induction of the low photorespiration state (Bowes et al., 1978; Ascencio, 1979; Salvucci and Bowes, 1981). Thus, there are at least two subgroups in regard to the major carboxylase enzyme in submersed macrophytes. In one of these

groups, PEP carboxylase activity may be quite high; however, plants in this group are not necessarily C_4 plants just because they exhibit high PEP carboxylase activity.

Other enzymes of C_4 acid metabolism. Several enzymes associated with C_4 acid metabolism also increase in activity upon induction of the low photorespiration state in Hydrilla, the only freshwater macrophyte for which information is available (Salvucci and Bowes, 1981). A similar increase in C_4 enzyme activities occurs in facultative CAM plants, upon induction of the CAM-mode (Kluge and Ting, 1978; Holtum and Winter, 1982). When considered in relation to the maximum photosynthetic rate (Van et al., 1976), the increased activities of C_4 enzymes upon induction of the low photorespiration state of Hydrilla are comparatively high. These enzymes include NAD-malic enzyme, malate dehydrogenase, aspartate aminotransferase and pyruvate Pi dikinase; the activities of which provide an increased enzymatic potential for C_4 acid decarboxylation, interconversion, and PEP regeneration in the low photorespiration state (Salvucci and Bowes, 1981). It is also possible that the activity of C_4 enzymes may also increase in other submersed macrophytes, specifically those in the Hydrocharitaceae (cf. De Groote and Kennedy, 1977; Browse et al., 1980), upon induction of the low photorespiration state (Browse et al., 1980; Salvucci and Bowes, 1981).

Carbonic anhydrase. Despite the preponderance of HCO_3^- in the aquatic environment, in only a few studies has the carbonic anhydrase activity of submersed macrophytes been measured. In freshwater species, carbonic anhydrase activity is considerably lower than in either emergent or terrestrial species (Van et al., 1976; Weaver and Wetzel, 1980; CHAPTER II), but this activity is probably in excess of that required for photosynthetic

HCO_3^- usage (Van et al., 1976). In Hydrilla and Myriophyllum, carbonic anhydrase activity increases by two- to three-fold upon induction of the low photorespiration state (CHAPTER II). An analogous increase in carbonic anhydrase activity occurs in unicellular green algae when transferred from high (5%) to low (0.03%) CO_2, although the increase is of a much greater magnitude in the algae (Graham et al., 1971; Hogetsu and Miyachi, 1979).

Relatively high levels of carbonic anhydrase activity have been reported in marine seagrasses (Graham and Smillie, 1976), although other investigators have found little or no activity in these plants (Beer et al., 1980). The low levels, however, may be due to the use of Tris-SO_4 in the extraction medium, as sulfonamides are potent inhibitors of carbonic anhydrase (Maren, 1967).

Photorespiratory enzymes. The activities of the enzymes of the photorespiratory carbon oxidation pathway in the few submersed macrophytes in which they have been measured coincide with the generally low activity of photosynthetic enzymes in these plants. Detailed information concerning the enzymatic capacity of the glycolate pathway in submersed macrophytes is not available since the activity of only two major enzymes, P-glycolate phosphatase and glycolate oxidase, has been measured and then only in a few species (Frederick et al., 1973; Stanley and Naylor, 1973; Van et al., 1976; Salvucci and Bowes, 1981). Glycolate dehydrogenase, an alternate glycolate oxidation enzyme in some algae, has also been assayed for a few freshwater macrophyte species, but activity has not been detected (Frederick et al., 1973; Salvucci and Bowes, 1981). Activity of this enzyme has been reported in extracts from two seagrass species, Cymodocea rotundata and Thalassia hemprichii, but it is not ubiquitous to seagrasses in that Halophila ovata possesses only glycolate oxidase

activity (Tolbert, 1976). The significance of glycolate dehydrogenase activity in some but not other marine angiosperms is unknown. Similarly, the significance of this enzyme in certain algae, where it replaces glycolate oxidase, is also an enigma. Glycolate dehydrogenase activity has not been reported in higher plants, from terrestrial environments, but there is insufficient data to speculate as to whether or not this enzyme may provide some advantage in an aquatic habitat.

Pathways of Photosynthetic Carbon Assimilation and Metabolism

Although it is clear that submersed macrophytes are not C_4 plants, in some species the C_4 acids, malate and aspartate can be major initial products of photosynthesis. Several species of freshwater macrophytes exhibit considerably greater C_4 acid fixation (20-50% of the initial products) than C_3 plants (cf. Rathnam and Chollet, 1979) and high levels of PEP carboxylase activity have been reported in many of these same species. Table 1 is a compendium of the results from a number of workers which lists for purposes of comparison the taxonomic status, the extent of C_4 acid versus C_3 product formation, the PEP carboxylase relative to RuBP carboxylase activity, and the polar versus nonpolar HCO_3^- use of several submersed aquatic macrophytes. Direct comparison of the extent of C_4 acid labeling reported by different investigators is not always possible since the conditions used for equilibration and ^{14}C fixation are usually not identical. For example, a greater fraction of the total label incorporated enters C_4 acids when the concentration of free CO_2 is reduced either by decreasing the total inorganic carbon concentration (Browse et al., 1979a; Holaday and Bowes, 1980) or by increasing the pH of the solution (Browse et al., 1979a). In addition, considerable variation in the percentage of C_4 acids formed may occur as a result of changes in the photorespiration state of the plant (Browse et al., 1980).

Table 1. Features associated with the occurrence of β-carboxylation in submersed aquatic macrophytes.

Species	β-Carboxylation Potential (+ or -)	Features	References
PTERIDOPHYTA			
Isoetaceae			
Isoetes sp.	+	net dark fixation, CAM-like malic acid levels	Keeley, 1981; Keeley and Bowes, 1982
SPERMATOPHYTA			
Dicotyledoneae			
Haloragaceae			
Myriophyllum brasiliense	-	high RuBP/PEP, variable PR	Salvucci and Bowes, 1982
Myriophyllum heterophyllum	-	high RuBP/PEP, variable PR, <10% C_4 acids	Salvucci and Bowes, 1981; Beer and Wetzel, 1982b
Myriophyllum spicatum	-	high RuBP/PEP, variable PR, <10% C_4 acids, nonpolar HCO_3^-	Stanley and Naylor, 1972; Salvucci and Bowes, 1981; Prins et al., 1982a
Ceratophyllaceae			
Ceratophyllum demersum	-	high RuBP/PEP, variable PR	Van et al., 1976; Salvucci and Bowes, 1981
Ranunculaceae			
Ranunculus baudotii	-	high RuBP/PEP	Valanne et al., 1982
Monocotyledoneae-freshwater			
Cyperaceae			
Scirpus subterminalis	+	low RuBP/PEP, C_4 acids, CAM-like malate levels	Beer and Wetzel, 1980, 1982b

Table 1 continued.

Species	β-Carboxylation Potential (+ or -)	Features	References
Hydrocharitaceae			
Egeria densa	+	low RuBP/PEP, C_4 acids, variable PR, polar HCO_3^-	Brown et al., 1974; Browse et al., 1980; Salvucci and Bowes, 1981; Prins et al., 1982a
Elodea canadensis	+	low RuBP/PEP, C_4 acids, polar HCO_3^-	Brown et al., 1974; De Groote and Kennedy, 1977; Prins et al., 1982a
Hydrilla verticillata	+	low RuBP/PEP, C_4 acids, variable PR, polar HCO_3^-, net dark fixation; CAM-like acid levels	Bowes et al., 1978; Holaday and Bowes, 1980; Salvucci and Bowes, 1981; Prins et al., 1982a
Lagarosiphon major	+	C_4 acids	Brown et al., 1974
Valisneria spiralis	+	C_4 acids, polar HCO_3^-	Helder and van Harmelen, 1982; Prins et al., 1982a
Najadaceae			
Najas flexilis	+	C_4 acids	Beer and Wetzel, 1982b
Potamogetonaceae			
Potamogeton praelongus	+	low RuBP/PEP, C_4 acids	Beer and Wetzel, 1982b
Potamogeton pectinatus	-	<10% C_4 acids	Winter, 1978
Monocotyledoneae-marine			
Hydrocharitaceae			
Halophila engelmanni	-	high RuBP/PEP, 10% C_4 acids	Aspden and Wells, 1980
Halophila spinulosa	-	<10% C_4 acids, C_3-labeling patterns	Andrews and Abel, 1979
Halophila stipulacea	+	low RuBP/PEP, C_4 acids	Beer et al., 1980
Thalassia hemprichii	?	conflicting reports of C_4 acids	Andrews and Abel, 1979; Beer et al., 1980
Thalassia testudinium	?	conflicting reports of C_4 acids	Benedict and Scott, 1976; Benedict et al., 1980; Beer and Wetzel, 1982a

Table 1 continued.

Species	β-Carboxylation Potential (+ or -)	Features	References
Potamogetonaceae			
Cymodocea nodosa	+	low RuBP/PEP, C_4 acids, C_4-labeling	Beer et al., 1980
Halodule uninervis	-	<10% C_4 acids	Beer and Waisel, 1979
Halodule wrightii	?	conflicting reports of C_4 acids	Aspden and Wells, 1980; Beer and Wetzel, 1982a
Syringodium isoetifolium	-	<10% C_4 acids	Beer and Waisel, 1979
Syringodium filiforme	?	conflicting reports of C_4 acids	Aspden and Wells, 1980; Beer and Wetzel, 1982a
Thalassodendron ciliatum	-	high RuBP/PEP, <10% C_4 acids	Beer et al., 1980
Zosteria marina	-	<10% C_4 acids	Beer and Wetzel, 1982a

Species are designated (+) if there is evidence for substantial C_4 acid metabolism or β-carboxylation and (-) if carbon assimilation is almost exclusively via the Calvin cycle. High or low RuBP/PEP refers to the ratio of the carboxylase activities. Unless otherwise stated, the presence of C_4 acids when indicated comprise from 15 to 60% of the ^{14}C incorporated.

Some dicotyledonous species of submersed freshwater macrophytes clearly lack the enzymatic potential for substantial β-carboxylation and appear to fix CO_2 almost exclusively via RuBP carboxylase-oxygenase (Table 1). One of these species, Myriophyllum spicatum, has been examined by several investigators and has been shown to possess both low levels of PEP carboxylase activity and pulse-chase labeling patterns that are similar to terrestrial C_3 plants, even when the plant is in the low photorespiration state (Salvucci and Bowes, 1981). It is interesting to note from Table 1 that dicotyledonous macrophytes, such as Myriophyllum, are nonpolar HCO_3^- users and non-C_4 acid formers while monocotyledonous, C_4 acid forming species that have been examined are recognized as polar HCO_3^- users. Polar or nonpolar HCO_3^- usage is no doubt a function of the characteristic differences in leaf anatomy between these submersed monocotyledonous and dicotyledonous species (Sculthorpe, 1971; Prins et al., 1982a). For this reason, there may also be a relationship among leaf anatomy, polar HCO_3^- usage and the occurrence of C_4 acid metabolism in submersed macrophytes (see discussion below).

The C_4 acids have been reported as major products of photosynthesis in some seagrass species, but not in others (Table 1). All seagrasses are monocotyledonous, and therefore the apparent relationship between monocots and C_4 acid formation found in freshwater species does not pertain to these marine angiosperms. Conflicting reports occur for several genera concerning the extent of C_4 acid fixation (Benedict and Scott, 1976; Andrews and Abel, 1979; Beer and Wetzel, 1982a). For example, values for C_4 acid labeling as high as 50% and as low as 6% have been reported by different workers for the seagrass Thalassia.. It is unknown whether these differences are due to different conditions used in the ^{14}C

fixation experiments (cf. Andrews and Abel, 1979) or perhaps because of natural variability such as found in freshwater species (Holaday and Bowes, 1980).

$\delta\ ^{13}C$ Values in Submersed Macrophytes

The ratio of $^{13}C/^{12}C$ is used with terrestrial plants to determine the principle pathway for carbon assimilation based on the fact that C_3 and C_4 plants exhibit differing capacities to discriminate between these two isotopes, largely because they possess different initial carboxylation enzymes (for a discussion of carbon isotope fractionation see O'Leary, 1981). Thus, $\delta^{13}C$ values for C_3 plants range from -23.2 to -34.3 o/oo, while C_4 plants fractionate to a lesser extent with values ranging from -11.5 to -18.3. Although the $\delta^{13}C$ values of several submersed macrophytes, especially seagrasses, have been determined, seldom do the values (-5.6 to -15.5 o/oo) correlate with data from labeling studies (Benedict and Scott, 1976; Andrews and Abel, 1979; Beer et al., 1980; Benedict et al., 1980; Smith and Walker, 1980). For example, C_4-like $\delta^{13}C$ values of -14.5 and -6.8 have been reported for two seagrass species that exhibit C_3-type labeling patterns (Andrews and Abel, 1979). It is now recognized that $^{13}C/^{12}C$ ratios of submersed macrophytes are influenced by factors which effect carbon isotope fraction that are peculiar to their environment and physiology. These factors include HCO_3^- utilization, large "unstirred" layers, variations in the $^{13}C/^{12}C$ ratio of the source water, and even the turbidity of the water. As a result, $\delta^{13}C$ values of submersed macrophytes are not strictly related to fractionation at the carboxylation reaction, but instead can be manifestations of isotope discrimination at other stages of carbon assimilation (Andrews and Abel, 1979; Smith and Walker,

1980; Osmond et al., 1981; Faquhar et al., 1982; Raven et al., 1982). Consequently, for submersed aquatic macrophytes, $\delta^{13}C$ values are not necessarily valid indicators of the carbon assimilation pathway. In this respect, they are similar to aquatic algae.

The Role of C_4 Acid Metabolism and Its Relationship to the Photorespiration State

On the basis of evidence from pulse-chase experiments, many investigators regard submersed aquatic macrophytes as C_3 plants (Hough and Wetzel, 1977; Beer and Waisel, 1979; Beer and Wetzel, 1982b; Helder and van Harmelen, 1982; Raven et al., 1982), including those which exhibit substantial β-carboxylation. This interpretation would appear to be erroneous because it ignores the possibility of dual carboxylation and also the potentially significant, but not readily apparent, contributions of malate and aspartate to photosynthetic carbon assimilation in several submersed macrophyte species. The turnover of label in submersed macrophytes is generally slower than in terrestrial plants (De Groote and Kennedy, 1977; Browse et al., 1980; Holaday and Bowes, 1980), and in general, if chase periods are of a relatively short duration (seconds), the fate of C_4 acids produced in photosynthesis cannot be determined (De Groote and Kennedy, 1977; Holaday and Bowes, 1980; Beer and Wetzel, 1982b).

From the few studies in which the chase period has been extended, there appear to be at least two possible patterns for malate metabolism in submersed species in the family Hydrocharitaceae, which exhibit substantial β-carboxylation:

1. The fraction of the label fixed into malate may be quite high during the initial pulse with ^{14}C and actually increase during an extended chase period of several hours with ^{12}C (Browse et al., 1980; CHAPTER III).

2. The high percentage of label initially fixed into malate may begin to turnover within the first minutes of an unlabeled chase period until almost all of the label is chased out of this compound within 30-60 minutes (De Groote and Kennedy, 1977; Holaday and Bowes, 1980; CHAPTER III).

In the first situation, malate acts more like an end-product of photosynthesis than an intermediate in that the malate pool expands only slowly in the light and is metabolized during the dark (Browse et al., 1980). Browse et al. (1980) suggest that this pattern of metabolism may be an indication that malate functions in these plants as an ion-balance to mediate cytoplasmic pH regulation or electroneutrality. The accumulation of malate during excess cation uptake is a widely-recognized phenomenon in a number of plant tissues (cf. Lüttge and Higinbotham, 1979). Recently it has been described in the Hydrocharitacean, submersed species, Valisneria spiralis, upon exposure to K_2SO_4 in the light (Helder and van Harmelen, 1982). Moreover, light-driven K^+ uptake is a major mechanistic feature of HCO_3^- utilization in those submersed macrophytes that have been examined (Lucas, 1980; Walker, 1980; Prins et al., 1982b), and it is thus likely that malate formation in these plants may be associated with their HCO_3^- utilization mechanism.

It is interesting that malate accumulates in the light for high photorespiration state Egeria and Hydrilla (the photorespiration state of Valisneria was not reported) but this C_4 acid quickly turns over in the light in Hydrilla plants in the low photorespiration state (Browse et al., 1980, CHAPTER III). It thus appears that Hydrilla plants in the low photorespiration state are representative of the second situation described above. The conditions in freshwater under which the low photorespiration state is induced are rather severe and include daytime pH values in excess

of 10 (Van et al., 1976). At this pH, free CO_2 levels are negligible and CO_3^{--}, an inhibitor of HCO_3^- usage in submersed macrophytes (Lucas, 1980; Walker, 1980; Prins et al., 1982a), replaces HCO_3^- as the predominant species of inorganic carbon in the environment. In general, CO_3^{--} ions cannot be used as a direct inorganic carbon source for photosynthesis (Raven, 1970). Thus, low photorespiration state plants, besides being exposed to conditions of low inorganic carbon availability, are also subjected to an environment in which HCO_3^- utilization is inhibited, and probably operates only to a very limited extent.

One possible adaptation to these daytime conditions of severely reduced carbon availability is dark fixation. This process has been shown to occur to a considerable degree in Hydrilla plants in the low but not the high photorespiration state (Holaday and Bowes, 1980), and also has been reported for the lower vascular plant Isoetes (Keeley, 1981) and the submersed angiosperm, Scirpus subterminalis (Beer and Wetzel, 1980). This situation is reminiscent of terrestrial CAM plants in which CO_2 is fixed in the dark into malate, and this C_4 acid is stored in the vacuole for decarboxylation during the day (Kluge and Ting, 1978). Several other CAM-like features can occur or be induced in these submersed macrophytes, including a diurnal rhythm in titratable acidity or malic acid levels and net CO_2 fixation in the dark (Beer and Wetzel, 1980; Holaday and Bowes, 1980; Keeley, 1981; Keeley and Bowes, 1982; Holaday et al., in press). Although speculative, it is quite possible that the induction of increased activity of C_4 acid enzymes that occurs in the low photorespiration state (Salvucci and Bowes, 1981) is largely for the purpose of metabolizing malate accumulated in the dark. The continued synthesis and turnover of malate in the light that is observed in the low photorespiration state

(CHAPTER III) suggests that, unlike in high photorespiration plants, malate formed during the day is rapidly metabolized by the high enzymatic capacity induce for matabolizing malate formed in the dark. Since high O_2 and temperature enhance photorespiratory CO_2 loss during conditions of low CO_2 availability, decarboxylation of C_4 acids formed in the light may be especially advantageous if the CO_2 derived from the C_4 acids can be accumulated internally to provide an endogenous source of inorganic carbon and reduce photorespiration (Salvucci and Bowes, 1981). This accumulation could conceivably be accomplished if outward diffusion of CO_2 is restricted by the resistance imposed by the large boundary layer due to the surrounding water. The method for formation and utilization of C_4 acids in the light is analogous to C_4 acid metabolism in C_4 plants and may explain the C_4-like gas exchange characteristics of the low photorespiration state in some macrophyte species (Bowes et al., 1978; Holaday and Bowes, 1980; Salvucci and Bowes, 1981).

Submersed Macrophytes with Low Potential for β-Carboxylation

Submersed freshwater macrophytes such as Myriophyllum are reminiscent of certain unicellular green algae in that they exhibit reduced photorespiration but no C_4 acid metabolism (Salvucci and Bowes, 1981). Reduced photorespiration in the algae is probably due to the operation of an electrogenic CO_2-concentrating mechanism which elevates the level of internal inorganic carbon (cf. Badger et al., 1980; Beardall and Raven, 1981). A similar type of CO_2-concentrating mechanism has been postulated as a mechanism for which to explain the low photorespiration state in non-C_4 acid forming submersed macrophytes (Salvucci and Bowes, 1981, 1982: Raven et al., 1982), based on the observation that their photorespiratory activity

is low when measured by gas exchange analysis. However, more recent data show that a reduced fraction of the label fixed in photosynthesis is shunted through photorespiratory intermediates (CHAPTER III), and this is more direct evidence that a true reduction in photorespiratory activity, as opposed to an enhancement of refixation, occurs in these plants. This reduction is repressed when carbonic anhydrase activity is inhibited; thus, as in algae, carbonic anhydrase is an important component of the photorespiration-reducing mechanism in Myriophyllum (CHAPTER III). An identical repression of the low photorespiration state is observed when carbonic anhydrase is inhibited in Hydrilla; a possible indication that aquatic plants require both elevated inorganic carbon and carbonic anhydrase in order to realize a change in the photorespiration state, regardless of the mechanism for carbon accumulation.

Concluding Statement

Although a renewed interest in the photosynthesis of submersed aquatic macrophytes has occurred in the last few years, available information pertaining to the physiological and biochemical details of photosynthetic carbon metabolism in these plants lags far behind our present state of knowledge for terrestrial plants. The variable nature of photorespiratory activity in submersed macrophytes is unique among higher plants, and offers many avenues for challenging future research, directed at investigating biochemical and environmental regulation of photosynthesis and photorespiration.

CHAPTER II
ETHOXYZOLAMIDE REPRESSION OF THE LOW PHOTORESPIRATION STATE IN SUBMERSED ANGIOSPERMS

Introduction

Nonsucculent terrestrial plants can be divided into categories based on their photosynthetic gas exchange characteristics. For C_3, C_4, and C_3-C_4 intermediate species, the ratio of photosynthesis/photorespiration is relatively constant within each category under a given set of measurement conditions (Chollet and Ogren, 1975; Rathnam and Chollet, 1980), except for minor changes during ontogeny (Tichá and Catský, 1981). In contrast, submersed freshwater angiosperms have been shown to exhibit very variable photosynthetic/photorespiratory activities dependent upon the conditions used for growth of the individual plants (Holaday and Bowes, 1980; Salvucci and Bowes, 1981; Holaday et al., 1982; Salvucci and Bowes, 1982). Submersed angiosperms incubated under a 30°C/14 h day exhibit high photosynthesis/photorespiration ratios as evidenced by low CO_2 compensation points, little inhibition of net photosynthesis by 21% O_2, increased net photosynthetic rates, and low rates of CO_2 evolution in the light (Salvucci and Bowes, 1981; Holaday et al., 1982; Salvucci and Bowes, 1982). Under a 12°C/10 h day, a high photorespiration state is induced as gas exchange characteristics of plants incubated under these conditions indicate a measurable level of photorespiratory activity (Salvucci and Bowes, 1981; Holaday et al., 1982; Salvucci and Bowes, 1982).

The induction of a continuum of high, through intermediate, to low photorespiration states distinguishes submersed angiosperms from nonsucculent terrestrial angiosperms which appear to be "locked" into a particular photorespiration state (Chollet and Ogren, 1975; Rathnam and Chollet, 1980); either high (C_3), low (C_4), or intermediate (C_3-C_4).

The inducibility of low or high photorespiration states for submersed angiosperms resembles a similar phenomenon reported for green algae (Graham et al., 1971; Badger et al., 1980; Beardall and Raven, 1981; Shelp and Canvin, 1981) and cyanobacteria (Badger et al., 1978). For these organisms, certain features of the photosynthetic process can be altered by the level of CO_2 used during growth. Growth under air-levels of CO_2, as opposed to 1-5% CO_2, results in an increase in the apparent affinity of photosynthesis for CO_2 and decreases in the degree of O_2 inhibition of photosynthesis and the rate of CO_2 evolution in the light (Badger et al., 1978; Tsuzuki and Miyachi, 1979; Beardall and Raven, 1981; Shelp and Canvin, 1981). These changes in gas exchange parameters have been attributed to the active accumulation of high levels of inorganic carbon by these organisms (Badger et al., 1978; Beardall and Raven, 1981), as evidenced by measurements of internal inorganic carbon concentrations approaching 1 mM for the green algae Chlamydomonas and Chlorella (Badger et al., 1980; Beardall and Raven, 1981) and over 4 mM for the cyanobacteria Anabaena and Coccochloris in the low-CO_2 grown state (Badger et al., 1978; Coleman and Colman, 1981). As these organisms are known to fix CO_2 primarily via ribulose bisphosphate (RuBP) carboxylase-oxygenase (Graham et al., 1971; Coleman and Colman, 1981) increasing the CO_2/O_2 ratio inside the cell could account for the observed gas exchange characteristics indicative of reduced photorespiration.

The enzyme carbonic anhydrase, which facilitates the interconversion of CO_2 and HCO_3^-, has been implicated in the inorganic carbon accumulation mechanism. Thus, levels of carbonic anhydrase activity are higher in algae and cyanobacteria grown at air-levels of CO_2 than when these organisms are grown at high CO_2 (Graham et al., 1971; Dohler, 1974; Hogetsu and Miyachi, 1979). Furthermore, treatment of air-grown algae with acetazolamide (Diamox) or ethoxyzolamide, inhibitors of carbonic anhydrase, suppresses the low photorespiration state, and cells thus treated physiologically resemble cells grown under high CO_2 (Graham et al., 1971; Badger et al., 1978; Tsuzuki and Miyachi, 1979). It was the purpose of this study to evaluate the involvement of carbonic anhydrase in the low photorespiration state of submersed angiosperms by examining the effect of ethoxyzolamide on various photosynthetic and photorespiratory activities. Two species were selected for comparison, Myriophyllum spicatum and Hydrilla verticillata, because previous work has indicated they exhibit biochemical differences in the low photorespiration state (Salvucci and Bowes, 1981).

Materials and Methods

Plant Species

Myriophyllum spicatum L. was collected from either Crystal River or the Suwanee River, Florida, and Hydrilla verticillata (L.F.) Royal was collected from Orange Lake near Cross Creek, Florida. Apical segments were grown in a solution containing 5% (v/v) Hoaglands solution under the conditions described by Holaday and Bowes (1980) to induce a high or low photorespiration state. Sorghum bicolor (L.) Moench, Nicotiana tabacum L. and Moricandia arvensis (L.) DC. were greenhouse grown.

Infrared Gas Analysis

For the submersed macrophytes, photosynthetic and dark respiratory activities were determined by measuring CO_2 uptake of evolution in a closed system as described previously (Salvucci and Bowes, 1981). For the terrestrial species, photosynthetic rates of detached leaves were calculated from the decrease in CO_2 concentration monitored in an opened system. Carbon dioxide compensation point values were used to verify the photorespiration state of each plant used in an experiment, and were determined as described by Van et al. (1976). All gas exchange measurements were performed at 30°C using a saturating quantum flux density of 1,000 $\mu E/m^2.s$. Gas exchange data presented in Figures 1-3 represent mean values from at least three separate determinations.

Ethoxyzolamide Treatments

For studies involving ethoxyzolamide, the aquatic plants were incubated and measured at 30°C in the light in a solution containing 10 mM 2-[N-morpholino] ethanesulfonic acid (Mes)-NaOH, 5% (v/v) Hoaglands solution, and 100 μM ethoxyzolamide at pH 5.5. Leaves from the terrestrial plants were detached under water and incubated with their cut end submerged in the ethoxyzolamide-containing solution. The incubation time was generally 4 hours, since longer incubation periods (24 hours) did not increase the ethoxyzolamide-stimulated effects.

Carbonic Anhydrase

Carbonic anhydrase (E.C. 4.2.1.1) assays were performed using 20 mM veronal buffer by the method of Hogetsu and Miyachi (1979). Plant extracts were prepared at 4°C by grinding 1.0 g of tissue in a Ten Broeck homogenizer with 5 ml of 25 mM tris(hydroxymethyl) aminomethane (tris)-HCl,

Figure 1. The effect of CO_2 concentration on the percent inhibition of net photosynthesis by 21% O_2 for Myriophyllum and Hydrilla in the low and high photorespiration states. The net photosynthetic rates were similar to those shown for low and high photorespiration Myriophyllum and low photorespiration Hydrilla in Figures 2 and 3, respectively.

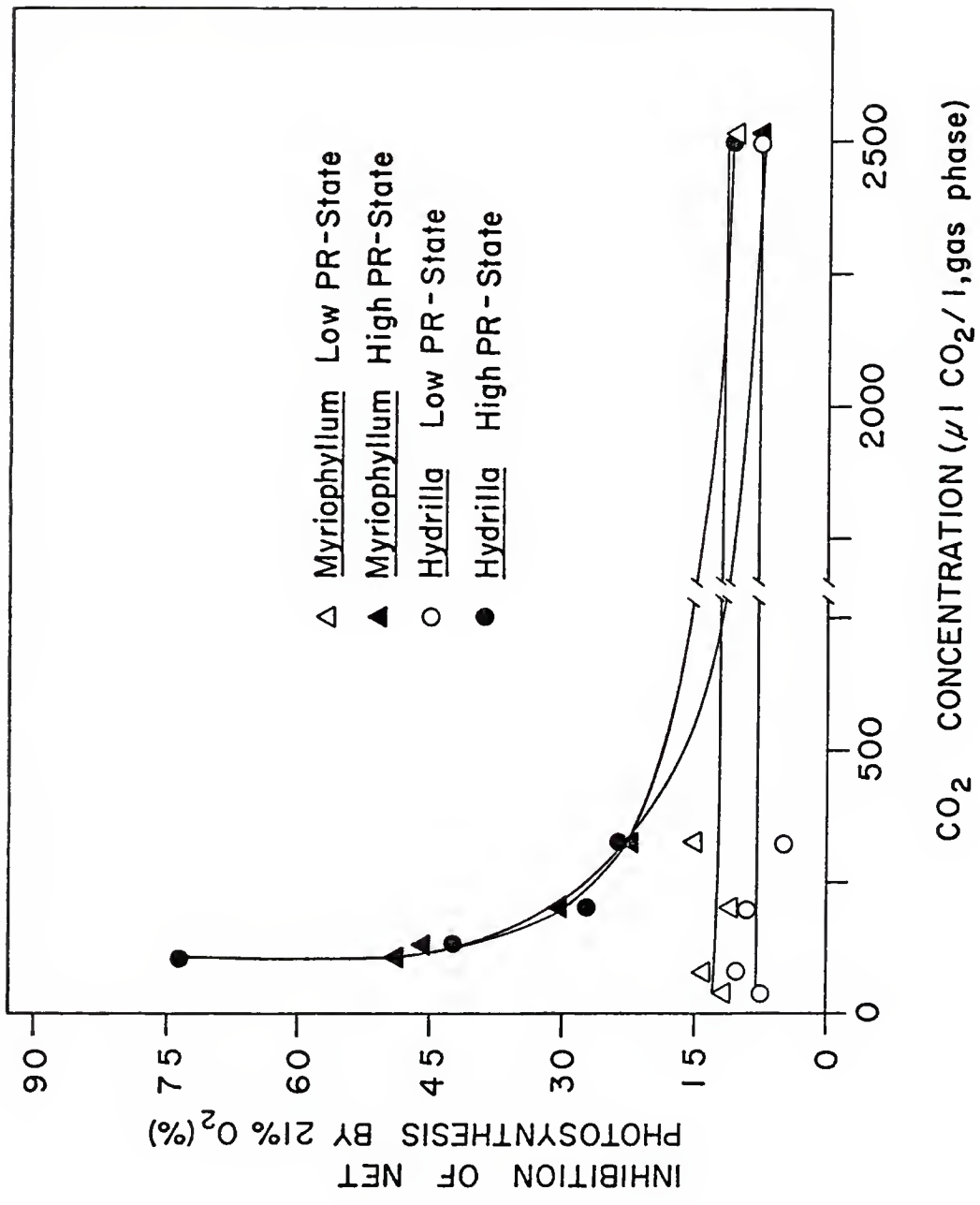

Myriophyllum Low PR-State
Myriophyllum High PR-State
Hydrilla Low PR-State
Hydrilla High PR-State
INHIBITION OF NET PHOTOSYNTHESIS BY 21% O2 (%)
0
15
30
45
60
75
90
0
500
2000
2500
CO2 CONCENTRATION (μl CO2/l, gas phase)

Figure 2. The effect of CO_2 concentration on net photosynthesis and percent inhibition of net photosynthesis by ethoxyzolamide for Myriophyllum in the low and high photorespiration state. Net photosynthetic rates were measured at 21% O_2 (gas phase).

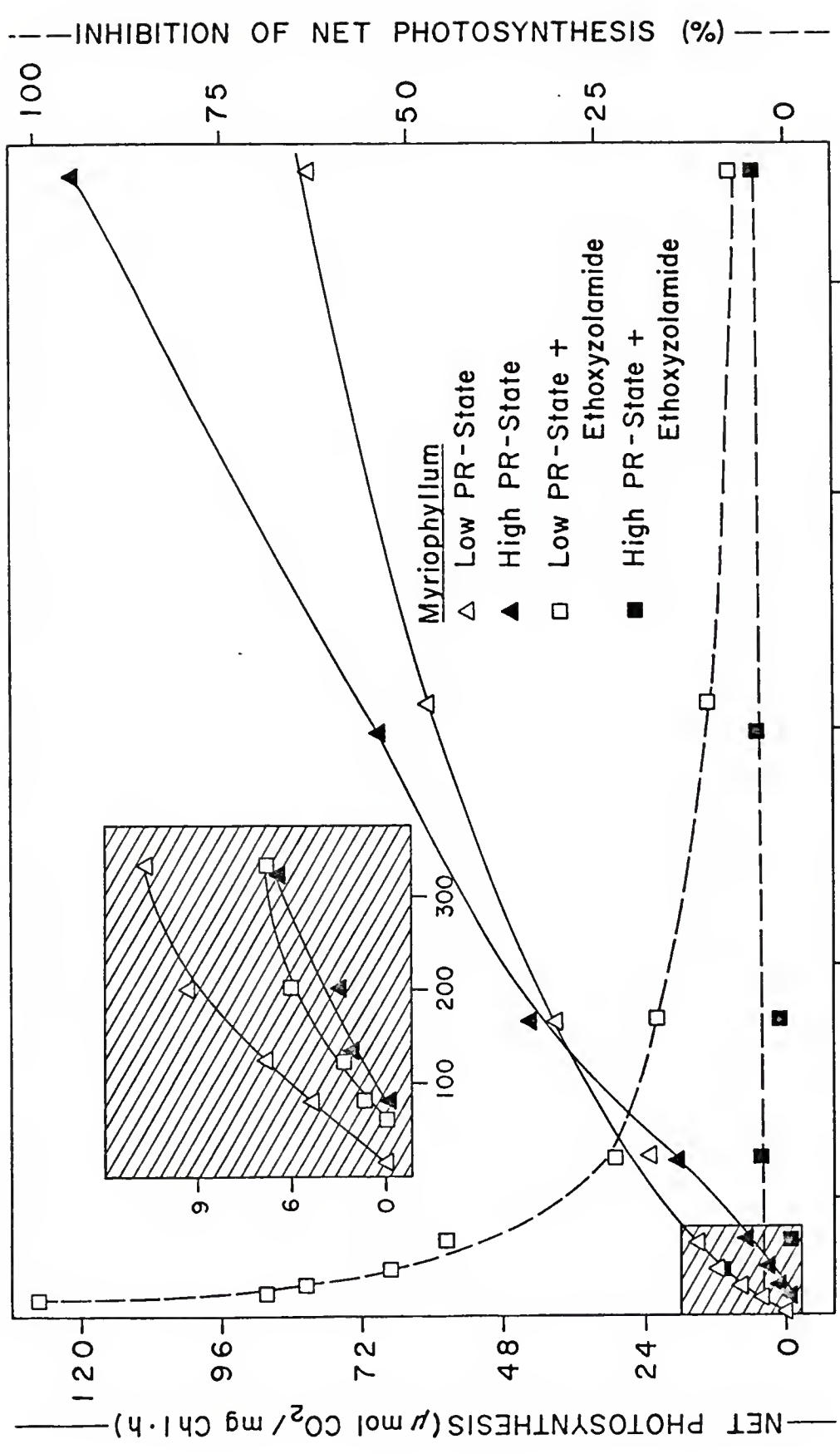
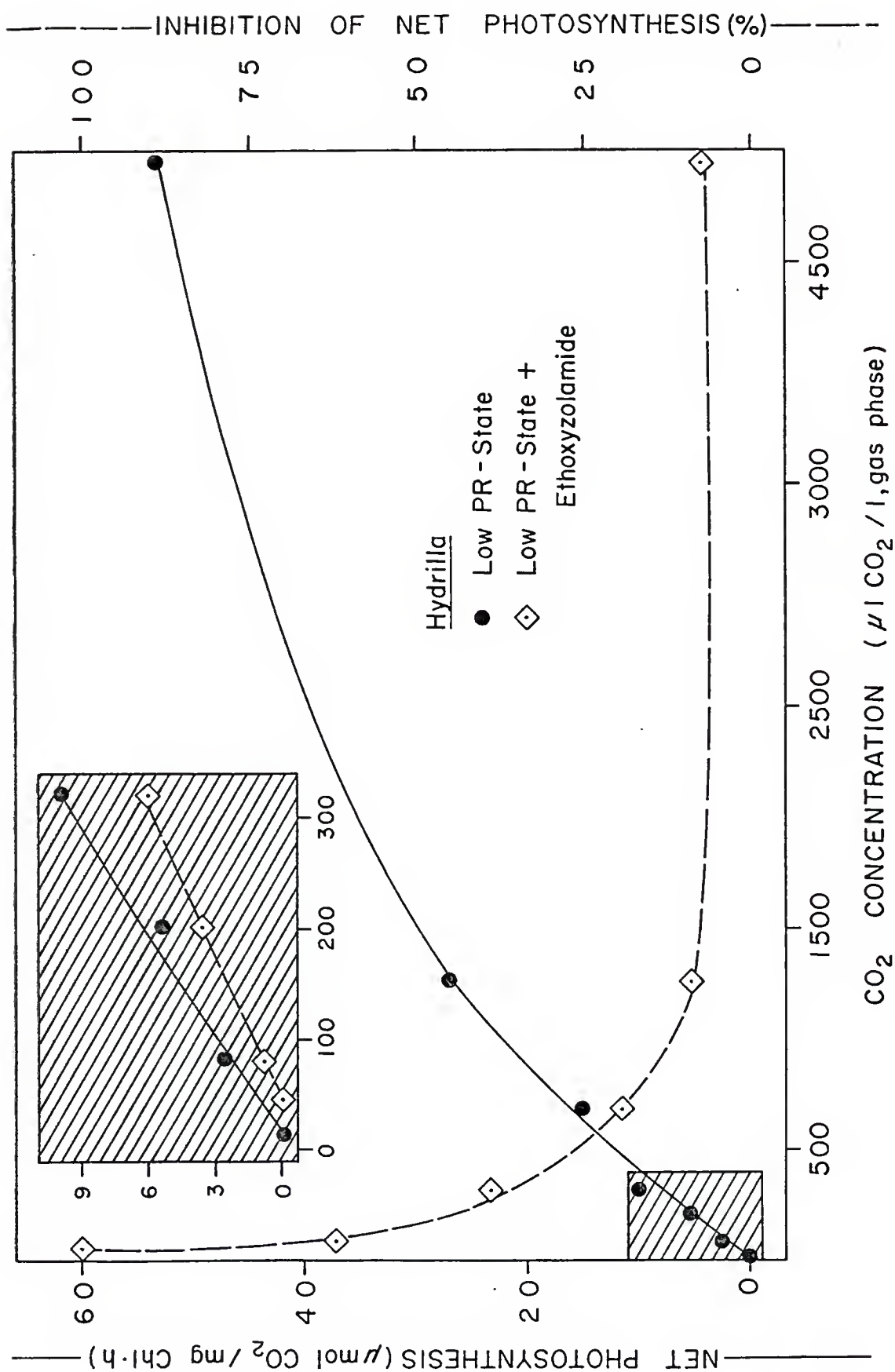

INHIBITION OF NET PHOTOSYNTHESIS (%)
100
75
50
25
0
Myriophyllum
Low PR-State
High PR-State
Low PR-State + Ethoxyzolamide
High PR-State + Ethoxyzolamide
NET PHOTOSYNTHESIS (μ mol CO2/ mg Chl·h)
120
96
72
48
24
0
500
1500
2500
3500
4500
CO2 CONCENTRATION (μl CO2/l, gas phase)
300
200
100
9
6
0

Figure 3. The effect of CO_2 concentration on net photosynthesis and percent inhibition of net photosynthesis by ethoxyzolamide for Hydrilla in the low photorespiration state. Net photosynthetic rates were measured at 21% O_2 (gas phase).


INHIBITION OF NET PHOTOSYNTHESIS (%)
100
75
50
25
0
Hydrilla
Low PR-State
Low PR-State + Ethoxyzolamide
NET PHOTOSYNTHESIS (μmol CO2 / mg Chl·h)
60
40
20
0
500
1500
2500
3000
4500
CO2 CONCENTRATION (μl CO2 / l, gas phase)
300
200
100
0
9
6
3
0

5 mM dithiothreitol (DTT), 0.1 mM ethylenediamine-tetraacetate (EDTA), and 2% (w/v) polyvinyl pyrrolidone (PVP)-40, pH 8.5. Extracts were filtered rapidly through 4 layers of cheesecloth and immediately assayed. Boiled extracts were used to determine the nonenzymatic rate of hydration. Preliminary experiments revealed the presence of an endogeous, heat-stable inhibitor of both the enzymatic and nonenzymatic activity in extracts from the aquatic plants. Filtration of the extracts through Sephadex G-25 failed to remove the inhibitor, but the inclusion of 2% (w/v) PVP-40 to the extraction and assay media eliminated inhibition. Enzyme activity is expressed as enzyme units (EU), where EU = $10(T_e/T_b-1)$ and T_e and T_b are the rates of enzymatic and nonenzymatic hydration, respectively.

PEP Carboxylase

Phosphoenolpyruvate (PEP) carboxylase (E.C. 4.1.1.31) was extracted and assayed as described previously by Van et al. (1976) except that 50 mM [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate] (Hepes)-KOH, pH 7.3 was used in the extraction medium. After extraction, the centrifuged extracts were freed of low molecular weight components by rapid filtration through Sephadex G-25 equilibrated with 25 mM Hepes-KOH and 10 mM $MgCl_2$, pH 7.2. As the eluants emerged from the columns, they were immediately supplemented with DTT to a final concentration of 10 mM and assayed.

Chlorophyll concentration was determined according to the method of Arnon (1949). Soluble protein was determined using the Coomassie Brilliant Blue dye method (Spector, 1978) with bovine serum albumin as a standard. The results presented for all the enzyme activities represent the mean values of triplicate determinations.

Results

The net photosynthetic rates for Myriophyllum and Hydrilla in the high and low photorespiration states were measured at 21 and 1% O_2 (gas phase), as a function of CO_2. The data are expressed as the percent inhibition due to the decrease in photosynthesis when measured at 21% O_2 as compared to 1% O_2 (Fig. 1). At air-saturated levels of CO_2 (330 µl/l, gas phase), the percent inhibition due to 21% O_2 was less for Myriophyllum and Hydrilla plants in the low photorespiration state (15.1 and 5.1%, respectively) than for those same plants in the high photorespiration state (22.6 and 23.8%, respectively). For the low photorespiration plants, changing the CO_2 level had no effect on the degree of O_2 inhibition (Fig. 1). In contrast, the O_2 inhibition in high photorespiration plants increased as the CO_2 concentration decreased (Fig. 1). Thus, at a CO_2 level of 106 µl CO_2/l, O_2 inhibition of photosynthesis was increased to 49 and 74%, respectively, for Myriophyllum and Hydrilla in the high photorespiration state, as compared with 8 and 11%, respectively, at 2,500 µl CO_2/l (Fig. 1).

An increase in carbonic anhydrase activity was associated with the low photorespiratory state of both Myriophyllum and Hydrilla. The activity of carbonic anhydrase was up to three-fold greater in the low photorespiration plants (Table 2). Hydrilla exhibited a substantially greater carbonic anhydrase activity than Myriophyllum when expressed on either a chlorophyll or protein basis (Table 2). The activities of this enzyme were considerably less in the submersed angiosperms, regardless of their photorespiration state, than in the terrestrial C_3 plant, tobacco (Table 2).

An inhibitor of carbonic anhydrase, ethoxyzolamide, at a concentration of 100 µM caused similar increases in the CO_2 compensation points of

Table 2. Carbonic anhydrase activity in Myriophyllum and Hydrilla in the low and high photorespiration (PR) states, and in Nicotiana.

Plant and PR state	Carbonic anhydrase activity (EU/mg Chl)	(EU/mg protein)
Myriophyllum		
Low PR	101	10
High PR	70	5
Hydrilla		
Low PR	916	39
High PR	342	18
Nicotiana	3408	118

Myriophyllum and Hydrilla in the low and intermediate photorespiration states (Table 3). An increase in the CO_2 compensation point was evident when measurements were made with 21 or 1% O_2 in the gas phase (Table 3). However, the increase was far greater for the CO_2 compensation points measured at 21% O_2. The effect was reversible upon removal of the ethoxyzolamide, and occurred, though to a lesser extent, with ethoxyzolamide concentrations as low as 20 μM (data not shown). The CO_2 compensation points of plants in the high photorespiration state, however, were unaffected by the ethoxyzolamide treatment (Table 3). Similarly, the CO_2 compensation points determined for the detached leaves of three terrestrial plants representing three distinct photosynthetic categories (C_4, C_3-C_4 intermediate, and C_3) were unaltered by ethoxyzolamide (Table 3). No effect on the compensation points were observable even after 12 hours incubation at twice the concentration used for the aquatic plants (200 μM). Apparently the inhibitor was taken up by these leaves, because after only a 4 hour exposure to 100 μM ethoxyzolamide the net photosynthetic rates were decreased by 3 to 11%.

When measured at 330 μl CO_2/l in the gas phase, the net photosynthetic rates of Myriophyllum and Hydrilla plants in the low photorespiration state were inhibited greatly by ethoxyzolamide (Table 4). Also, at this CO_2 concentration, the O_2 inhibition of photosynthesis was increased two- to four-fold for the low photorespiration plants by the presence of the inhibitor (Table 4). In contrast, for Myriophyllum and Hydrilla plants in the high photorespiration state, net photosynthesis and O_2 inhibition were far less affected by ethoxyzolamide (Table 4). The rates of dark CO_2 evolution (respiration) for the low photorespiration plants were not altered by the inhibitor (Table 4).

Table 3. Effect of ethoxyzolamide on the CO_2 compensation points of Myriophyllum, Hydrilla, and Proserpinaca in different photorespiration (PR) states, and of Sorghum, Moricandia, and Nicotiana.

Plant type and PR state	CO_2 compensation point (μl CO_2/l)			
	Control		Ethoxyzolamide	
	21% O_2	1% O_2	21% O_2	1% O_2
Myriophyllum				
Low PR	14	7	47	18
Intermediate PR	37	15	64	-
High PR	81	55	81	55
Hydrilla				
Low PR	14	7	41	25
Intermediate PR	48	29	70	-
High PR	91	60	98	65
Proserpinaca[a]				
Low PR	7	7	45	26
Sorghum				
C_4 species	3	-	4	-
Moricandia				
C_3-C_4 species	22	2	21	1
Nicotiana				
C_3 species	60	10	61	8

[a]Emergent form incubated and measured under water.

Table 4. Effect of ethoxyzolamide on net photosynthesis at 21 and 1% O_2 and 330 µl CO_2/l, and on dark CO_2 evolution for Myriophyllum and Hydrilla in the low and high photorespiration (PR) states.

Plant and PR state	Treatment	Net photosynthesis (µmol CO_2/mg Chl·h)		Inhibition by 21% O_2 (%)	Dark CO_2 evolution (µmol CO_2/mg Chl·h)
		21% O_2	1% O_2		
Myriophyllum					
Low PR	Control	15.3 ± 3.9[a]	17.7 ± 5.1	13.6	6.8 ± 0.9
	Ethoxyzolamide	9.7 ± 3.6	12.9 ± 5.1	24.8	7.5 ± 1.4
High PR	Control	7.1 ± 0.7	9.2 ± 0.7	22.8	-
	Ethoxyzolamide	7.1 ± 0.8	9.0 ± 1.0	21.1	-
Hydrilla					
Low PR	Control	7.1 ± 1.1	7.4 ± 1.2	4.1	2.0 ± 0.1
	Ethoxyzolamide	4.0 ± 0.4	4.8 ± 0.5	16.7	1.8 ± 0.2
High PR	Control	4.8 ± 0.9	6.7 ± 0.4	28.4	-
	Ethoxyzolamide	4.3 ± 0.4	6.9 ± 0.4	37.7	-

[a]Mean ± standard deviation.

As shown in Figure 2, for Myriophyllum plants in either photorespiration state, net photosynthesis could be increased by raising the CO_2 concentration, but saturation was not achieved even at 5,000 µl CO_2/l. Treatment of the low photorespiration Myriophyllum plants with 100 µM ethoxyzolamide substantially inhibited photosynthetic CO_2 fixation (Fig. 2 inset), as previously indicated in Table 4. However, the percent inhibition of photosynthesis caused by the ethoxyzolamide treatment could be decreased by raising the CO_2 concentration (Fig. 2). Myriophyllum plants in the contrasting high photorespiration state exhibited only a 5% inhibition of photosynthetic CO_2 fixation as a result of the ethoxyzolamide treatment, and this small degree of inhibition was independent of the CO_2 concentration (Fig. 2). In a related experiment, the photosynthetic rates of detached leaflets of low photorespiration Myriophyllum were determined in terms of O_2 evolution. The data obtained were similar to that shown in Figure 2, in that at pH 8, photosynthetic O_2 evolution was decreased by ethoxyzolamide at sub-saturating, but not at saturating, HCO_3^- levels (data not shown).

The data in Figures 2 and 3 produced linear responses, when transformed into double reciprocal plots. From an evaluation of these data in terms of Michaelis-Menton kinetics, estimates of the apparent $Km(CO_2)$ values for photosynthesis were calculated using the statistical methods of Wilkinson (1961). This provided an approximation of the apparent affinity of the plants for CO_2. For high and low photorespiration Myriophyllum, the apparent $Km(CO_2)$ values for photosynthesis were calculated as 175 and 64 µM, respectively. When low photorespiration Myriophyllum plants were measured in the presence of 100 µM ethoxyzolamide, their apparent photosynthetic affinity for CO_2 decreased to a level approaching

that of high photorespiration plants, as indicated by a rise in the apparent $Km(CO_2)$ to 123 μM. No increase in the apparent $Km(CO_2)$ for photosynthesis occurred for high photorespiration Myriophyllum plants treated with ethoxyzolamide.

Net photosynthesis for low photorespiration Hydrilla increased with increasing CO_2 concentration, but as with Myriophyllum, 5,000 μl CO_2/l did not saturate photosynthesis (Fig. 3). An inhibitory effect of 100 μM ethoxyzolamide on net photosynthesis similar to that observed for low photorespiration Myriophyllum was evident for Hydrilla plants in the low photorespiration state, and this inhibition could similarly be relieved by increasing the CO_2 concentration (Fig. 3). Calculation of the apparent $Km(CO_2)$ value for photosynthesis in low photorespiration Hydrilla indicated it increased from 74.7 to 90.2 μM in the presence of ethoxyzolamide.

Because Hydrilla plants in the low photorespiration state utilize PEP carboxylase as a major carboxylation enzyme (Holaday and Bowes, 1980), the effect of ethoxyzolamide on the in vitro activity of this enzyme from Hydrilla was examined. The activity of PEP carboxylase was 120 μmol/mg Chl·h, at saturating substrate concentrations, and 100 μM ethoxyzolamide had no inhibitory effect on this rate. Similarly, 100 μM ethoxyzolamide had no effect on the enzyme rate when the enzyme was assayed under limiting substrate levels of 0.4 mM HCO_3^- or 0.5 mM PEP.

Discussion

It has been well documented for terrestrial C_3 plants that the O_2 inhibition of net photosynthesis is increased at low CO_2 concentrations (Chollet and Ogren, 1975). Substantial evidence indicates that this

phenomenon is due to the competitive interactions between CO_2 and O_2 at the active site of RuBP carboxylase-oxygenase (Chollet and Ogren, 1975). The degree of O_2 inhibition of net photosynthesis and its sensitivity to CO_2 suggests that gas exchange measurements with Myriophyllum and Hydrilla in the high photorespiration state also reflect the properties of RuBP carboxylase-oxygenase. Thus in the high photorespiration state, these aquatic angiosperms, like terrestrial C_3 plants, apparently lack an effective mechanism to insulate the initial events of photosynthetic and photorespiratory carbon metabolism from external CO_2 and O_2 concentrations. In contrast, the low level of O_2 inhibition and its insensitivity to CO_2 observed with Myriophyllum and Hydrilla in the low photorespiration state imply that some type of photorespiration-reducing mechanism is induced in these plants (Salvucci and Bowes, 1981; Holaday et al., 1982; Salvucci and Bowes, 1982).

Several photosynthetic characteristics found in Myriophyllum and Hydrilla upon induction of the low photorespiration state resemble those reported for unicellular green algae. For example, the induction of the low photorespiration state in Myriophyllum and Hydrilla results in increased carbonic anhydrase activity; a similar phenomenon has been reported for algal cells transferred from high (5%) CO_2 to low (air-levels) CO_2 (Graham et al., 1971; Badger et al., 1978; Hogetsu and Miyachi, 1979). Furthermore, ethoxyzolamide treatment decreases the net photosynthetic rate but increases the apparent $Km(CO_2)$ for photosynthesis, the O_2 inhibition of photosynthesis, and the CO_2 compensation points of low photorespiration Myriophyllum and Hydrilla. Similar results have been reported for low CO_2-grown Chlorella and Chlamydomonas cells (Tsuzuki and Miyachi, 1979; Badger et al., 1980). The competitive nature of increasing CO_2 levels

decreasing the ethoxyzolamide inhibition of photosynthesis also appears to be common to these aquatic angiosperms and unicellular green algae (Tsuzuki and Miyachi, 1979; Badger et al., 1980). As in the green algae, all of these results point to the involvement of carbonic anhydrase in the photorespiration-reducing mechanism of both Myriophyllum and Hydrilla. Rather than the sole factor, carbonic anhydrase in Myriophyllum and Hydrilla appears to be but one component of the photorespiration-reducing mechanism, in that the low CO_2 compensation points of plants in the low photorespiration state were increased to the level of those in the intermediate state but not to those of the high photorespiration plants.

Although carbonic anhydrase has been implicated in the green algae, the observed photosynthetic characteristics of low CO_2-grown algal cells appear to be due to an elevated level of inorganic carbon at the site of fixation by RuBP carboxylase-oxygenase, rather than to carbonic anhydrase activity per se. Thus, by measuring the internal inorganic carbon concentrations in Chlamydomonas, Badger et al. (1980) showed that ethoxyzolamide decreased the rate but not the final concentration of inorganic carbon accumulated. Recently, Spalding et al. (1982) have isolated high CO_2-requiring mutants of Chlamydomonas, one deficient in carbonic anhydrase activity but capable of accumulating high levels of inorganic carbon, and the other unable to accumulate inorganic carbon despite normal levels of carbonic anhydrase. The physiological phenotypes of both mutants resemble those of high CO_2-grown wild type Chlamydomonas cells indicating that inorganic carbon transport/accumulation and carbonic anhydrase activity are two separate components, both of which are required for the low photorespiration (low CO_2-grown) characteristics. Membrane hyperpolarization studies with the green algae Chlorella (Beardall, 1981; Beardall and

Raven, 1981) and the cyanobacterium Anabaena (Kaplan et al., 1982), using the lipophilic cationic probe tetraphenyl phosphonium (TPP^+), have characterized the inorganic carbon accumulation component of the photorespiration-reducing mechanism as an electrogenic HCO_3^- pump located either at the plasmalemma (Kaplan et al., 1982) or possibly on the chloroplast membrane (Beardall, 1981; Beardall and Raven, 1981).

To date, all higher plants and algae with reduced photorespiratory capacity utilize mechanisms that increase the CO_2 concentration at the site of RuBP carboxylase-oxygenase (Chollet and Ogren, 1975; Rathnam and Chollet, 1980; Beardall and Raven, 1981). For cyanobacteria, there is evidence to suggest that the very high levels of internal inorganic carbon (4 mM) can be utilized for photosynthesis without increased carbonic anhydrase activity (Badger et al., 1978). In the green algae, however, it appears that carbonic anhydrase is needed to facilitate the rapid equilibration of the inorganic carbon forms in order for these organisms to effectively use the elevated internal levels of inorganic carbon as a concentrated source of free CO_2 (Badger et al., 1980). An equilibration function for carbonic anhydrase may also be required for Myriophyllum and Hydrilla to achieve the low photorespiration state.

The biochemical basis for the reduced photorespiration state of submersed aquatic macrophytes involves at least two different mechanisms. For Hydrilla, low photorespiration is associated with the induction of increased C_4 acid metabolism (Holaday and Bowes, 1980; Salvucci and Bowes, 1981), and it now appears likely that decarboxylation of the malate pool results in elevated internal inorganic carbon levels analogous to the situation in terrestrial C_4 plants (CHAPTER III). Despite the likely use by Hydrilla of a C_4 system to elevate the CO_2, the data in this study

indicate that carbonic anhydrase is still a component required to effectively maintain the low photorespiration state. In the high photorespiration state of Hydrilla, the function of carbonic anhydrase in photosynthesis is far less apparent.

In contrast to Hydrilla, Myriophyllum in the low photorespiration state exhibits little phosphoenolpyruvate carboxylase activity and C_4 acids account for less than 10% of the initial photosynthetic products (Salvucci and Bowes, 1981; CHAPTER III). Thus, a C_4 acid system for elevating internal CO_2 levels cannot be operative in Myriophyllum. Whether an algal-like electrogenic HCO_3^- pump together with the elevated activity of carbonic anhydrase function as components of the Myriophyllum system is presently unclear. Evidence for active HCO_3^- utilization mechanisms have been presented for a number of submersed freshwater macrophytes including Myriophyllum and Hydrilla (Lucas, 1980; Walker, 1980; Prins et al., 1982a). Consequently, although free CO_2 generally appears to be the preferred form for the photosynthesis of submersed angiosperms (Van et al., 1976), the internal accumulation of significant amounts of inorganic carbon by an electrogenic HCO_3^- pump, which in Myriophyllum would be induced or increased in the low photorespiration state, cannot be ruled out.

The lack of an ethoxyzolamide-stimulated effect on the CO_2 compensation point of Sorghum suggests that in terrestrial C_4 plants, the utilization of elevated inorganic carbon levels to overcome photorespiration in the bundle sheath does not depend on the activity of carbonic anhydrase. In a similar manner, the CO_2 compensation point was not increased in the C_3-C_4 intermediate Moricandia or in the C_3 plant tobacco, a possible indication that the involvement of carbonic anhydrase in maintaining a particular photosynthesis/photorespiration ratio, at least at levels of CO_2 near the CO_2 compensation point, may be restricted to aquatic plants.

CHAPTER III
TWO PHOTOSYNTHETIC MECHANISMS MEDIATING THE LOW PHOTORESPIRATION STATE IN SUBMERSED AQUATIC ANGIOSPERMS

Introduction

It has become a common practice to differentiate among plants on the basis of their photosynthetic and photorespiratory carbon metabolism. Thus, terrestrial C_3 and C_4 plants can be distinguished on the biochemical basis of their first products of carbon fixation. However, they concomitantly exhibit characteristic physiological and ecological differences, especially in regard to gas exchange (Chollet and Ogren, 1975). Consequently, the terms C_3 and C_4 take on a much broader meaning when they are used to describe the complete syndrome of biochemical and physiological characteristics associated with each group. Recently some investigators have made use of this more descriptive interpretation in applying the term C_3-C_4 intermediate species to terrestrial plants which exhibit gas exchange characteristics, anatomical features, and, for some species, levels of C_4 acid metabolism, which are intermediate between C_3 and C_4 plants (Rathnam and Chollet, 1980; Holaday et al., 1982). In the same vein, the term CAM (Crassulacean Acid Metabolism) has come to refer to a cadre of photosynthetic characteristics, found largely in succulent plants, and not just to the biochemical mechanisms involved (Kluge and Ting, 1978).

Aquatic autotrophs pose a problem in regard to categorization. Certain unicellular green algae and cyanobacteria, such as Chlorella (Beardall and Raven, 1981; Shelp and Canvin, 1981), Chlamydomonas (Badger et al., 1978), and Anabaena (Badger et al., 1978), exhibit changeable photosynthetic and photorespiratory characteristics depending upon the CO_2 concentration at which they are grown. Thus on the basis of their gas exchange features, these algae do not fit into either the C_3 or C_4 category. Based on their first product of carbon fixation they have been referred to as "C_3 plants" (Beardall and Raven, 1981), however, this designation becomes confusing for low CO_2-grown algae which physiologically are C_4-like, and thus could be described as C_4-like C_3 plants (Beardall and Raven, 1981). Unicellular green algae and cyanobacteria also differ from C_3-C_4 intermediates in that the algae appear to exhibit a continuum of photorespiration states and not a fixed intermediate level as found in terrestrial C_3-C_4 intermediate species (Badger et al., 1980).

Submersed freshwater angiosperms also exhibit a continuum of photorespiratory states, ranging from high to low, and in this regard are similar to unicellular green algae (Bowes et al., 1978; Salvucci and Bowes, 1981). However, the difficulties encountered in assigning green algae to the C_3 or C_4 categories are complicated further in submersed angiosperms by evidence that C_4 acids can be major products of photosynthesis in some, but not all, aquatic macrophytes (Stanley and Naylor, 1972; Holaday and Bowes, 1980; Beer and Wetzel, 1982b); even in those with considerable photorespiratory activity (Browse et al., 1980). In addition, features such as dark ^{14}C fixation, net fixation of CO_2 in the dark, and diurnal fluctuations in the level of titratable acidity, which are generally associated with CAM plants (Kluge and Ting, 1978), have been

observed in certain submersed aquatic macrophytes (Holaday and Bowes, 1980; Beer and Wetzel, 1981; Keeley, 1981; Keeley and Bowes, 1982; Holaday et al., 1982). Thus, photosynthetic carbon metabolism in submersed aquatic macrophytes can involve a combination of features, each generally thought to be characteristic of a particular photosynthetic category (i.e., C_3, C_4, C_3-C_4 intermediate, or CAM).

Part of the difficulty in categorizing submersed angiosperms is due to the fact that it is not completely understood how the various pathways for carbon assimilation relate to the level of photorespiratory activity or to its variable nature (Browse et al., 1980; Holaday and Bowes, 1980). A ^{14}C labeling study using the submersed angiosperm Myriophyllum indicates that carbon assimilation in this plant is via the Calvin cycle (Stanley and Naylor, 1972), however, the photorespiration state of the plants used in the study was not determined. Reportedly low levels of PEP carboxylase activity provide further evidence that CO_2 fixation in Myriophyllum is predominately via the Calvin cycle, even when the plant is in the low photorespiration state (Salvucci and Bowes, 1981, 1982). In contrast, from previous studies with Hydrilla, it appears that C_4 acids are major early products of photosynthesis and that the induction of the low photorespiration state in Hydrilla is associated with an increase in the activities of several enzymes involved in C_4 acid metabolism (Holaday and Bowes, 1980; Salvucci and Bowes, 1981). The formation of C_4 acids in photosynthesis has been reported for several other submersed macrophyte species but,as in Hydrilla, their role in photosynthetic carbon metabolism is unclear (Brown et al., 1974; De Groote and Kennedy, 1977; Beer and Wetzel, 1982b). Pulse-chase labeling experiments have not resolved this question in that the turnover of label fixed in malate is

reported either not to occur (Browse et al., 1980), or to turn over at a slower rate during the chase period than in terrestrial C_4 species (De Groote and Kennedy, 1977; Holaday and Bowes, 1980).

In order to clarify some of these problems, the relationship between C_4 acid metabolism and the variable photorespiration states in Hydrilla was examined especially with regard to the fate of malate produced in photosynthesis. By way of comparison, the intermediates of photosynthetic carbon metabolism in Myriophyllum were also investigated. Because a low photorespiration state is inducible in Myriophyllum, as well as in Hydrilla, an attempt was made to further elucidate the mechanism(s) responsible for this state. This was accomplished by examining the interactive effects of O_2; CO_2; ethoxyzolamide, which is a repressor of the low phororespiration state (CHAPTER II); and isonicotinic acid hydrazide, which inhibits carbon flow through the photorespiratory pathway (Servaites and Ogren, 1977); on the gas exchange characteristics and on the relative fluxes of carbon through the photorespiratory pathways of these plants.

Materials and Methods

Plant Material

Myriophyllum spicatum L. and Hydrilla verticillata (L.F.) Royal were collected and incubated as described in CHAPTER II.

Infrared Gas Analysis

Net photosynthetic rates were determined by measuring CO_2 uptake in a closed system as described previously (Salvucci and Bowes, 1982). Carbon dioxide compensation point values were used to verify the photorespiration state of each plant used in an experiment, and were determined

as described by Van et al. (1976). The CO_2 compensation point has been shown to be a reliable indicator of the photorespiration state (Salvucci and Bowes, 1981). All gas exchange measurements were performed at 30°C using a saturating quantum flux density of 1,000 $\mu E/m^2 \cdot s$. Gas exchange data represent mean values from at least three separate determinations.

Initial Product and Pulse-Chase Experiments

Carbon fixation experiments were performed at 30°C in the light under a saturating quantum flux density of 900 $\mu E/m^2 \cdot s$ (400-700 nm). After a 1-2 h equilibration period, apical segments of Myriophyllum and Hydrilla, 5 to 10 cm in length, were transferred from an aerated (317 μl CO_2/l and 21% O_2, gas phase) solution of 10 mM Mes-NaOH and 5% Hoaglands solution at pH 5.5 (Mes-Hoaglands solution) and pulsed for 20 s in 200 ml of Mes-Hoaglands solution containing 20 μM $NaH^{14}CO_3$ (50 $\mu Ci/\mu mol$). Prior to the addition of $H^{14}CO_3^-$, the pulse solution was purged for 1 h with CO_2-free air. In pulse-chase experiments, the labeled plants were transferred to a second aerating solution to complete the duration of the chase. Aeration established a free CO_2 concentration of 9.0 μM for the chase portion of the experiment. Carbon fixation was stopped by rapidly plunging the plants into liquid N_2.

Experiments involving ethoxyzolamide employed the above procedures except that ethoxyzolamide at 100 μM was included in all solutions, and the equilibration period was extended to 4 h (CHAPTER II).

Glycine Accumulation

Carbon fixation experiments were performed in a shaking water bath using detached leaves of Myriophyllum and Hydrilla. The light and temperature conditions were as described above. Entire apical segments were

incubated at 25°C in the dark for 1 h in Mes-Hoaglands solution containing various isonicotinic acid hydrazide (INH) concentrations. After 1 h, 2 leaves (approximately 50 μg Chl) were detached and added to 25 ml reaction flasks with 10 ml of the INH-containing solution, which had been previously purged for 20 min with a CO_2-free N_2/O_2 mixture. Purging was continued for 7 min in the light to equilibrate the leaves, after which time the flasks were rapidly sealed and fixation initiated by the addition of 0.1 ml $NaH^{14}CO_3$ (50 μCi/μmol) through a serum cap. After 15 min, carbon fixation was stopped by rapidly plunging the leaves into liquid N_2. Experiments were run in duplicate and, for each experiment, the pooled contents from two replicate vials were analyzed.

Extraction of Labeled Compounds

The plant material frozen in liquid N_2 was ground in a Ten Broeck homogenizer containing 80% (v/v) acetone at 4°C. The resulting homogenate was quantitatively transferred to a 15 ml centrifuge tube and aliquots were taken to determine chlorophyll content (Arnon, 1949) and total ^{14}C fixed. For the latter, aliquots were added to scintillation vials containing 0.5 ml H_2O acidified with 0.1 ml of a 6 N HCl solution saturated with 2,4-dinitrophenylhydrazine (DNPH). Triplicate determinations for each sample were taken to dryness and ^{14}C dpm were determined by liquid scintillation spectrometry. Eighty-five percent ethanol containing 0.12% (w/v) DNPH was added to the remainder of the homogenate and the resulting mixture was warmed to room temperature. After 1 h, the solution was centrifuged for 20 min at 10,000 g and the pellet was extracted sequentially with boiling solutions of ethanol (85%, 40%, and 20%), distilled H_2O (twice) and 0.5 N formic acid, each for 10 min. The supernatants from each extraction were pooled and taken to dryness overnight at 40°C

under a stream of air. The amount of ^{14}C label remaining in the pellet was determined and represented the insoluble fraction. The supernatant fraction was reconstituted with H_2O and then extracted twice with chloroform to remove lipids and unreacted DNPH, and finally lyophilized. The ^{14}C in the chloroform fraction was generally less than 1.0% of the total ^{14}C fixed. Determinations of radioactivity in the chloroform, insoluble and supernatant fractions indicated that greater than 93% of the total ^{14}C fixed was recovered at this stage.

Separation of Labeled Compounds

Following the reconstitution of the lyophilate in H_2O, the extract was quantitatively fractionated by ion-exchange chromatography (Atkins and Canvin, 1971). Basic compounds were eluted from an 8.0 x 60.0 mm column of Dowex 50X8-400 with 2 N NH_4OH. Nonbasic compounds were separated into neutral, acid 1 and acid 2 fractions by chromatography on Dowex 1X8-400, eluted with H_2O, 3 N formic acid, and 4 N HCl, respectively. These elution conditions, as confirmed by the separation of authentically labeled standards (^{3}H-glucose, ^{14}C-malate, ^{14}C-P-glycerate, ^{14}C-fructose 1,6 bisP), provided virtually complete separation of malate from P-glycerate. The ^{14}C dpm were determined for each fraction and recovery of ^{14}C from the ion-exchange columns was nearly 100% as has been reported by other workers (Atkins and Canvin, 1971). Each fraction from the columns was lyophilized and then reconstituted by the addition of 100 µl of 10% ethanol, with the exception of the neutral fraction to which 100 µl of H_2O was added.

Thin-Layer Chromatography

Individual compounds in each ion-exchange fraction were separated by one-dimensional chromatography on plastic backed thin-layer plates (MN-Cellulose 300, Brinkmann Instruments, Westbury, New York, U.S.A.). The plates were developed twice in the appropriate solvent system after overspotting five 2 µl aliquots of each sample using a capillary pipet. Aliquots of each sample were also added directly to scintillation vials with the 2 µl capillary pipet to determine the total ^{14}C added to the plate prior to separation. Recovery of ^{14}C from the thin-layer plates, after separation, exceeded 95%. In the basic fraction, amino acids were separated in n-butanol-acetone-water-diethylamine (20:20:15:3). Compounds in the acid 1 fraction were separated in ethanol-ammonium hydroxide-water (6:1:1.6, equilibrated for 24 h) and in sec-butanol-formic acid-water (6:1:2). This latter solvent system was also used for the acid 2 fraction. The neutral fraction was separated by chromatography in n-butanol-acetic acid-water (12:3:5). Individual compounds were localized by co-chromatography with authentic standards, and areas corresponding to these markers were cut out and their ^{14}C dpm determined. Authentic amino acids, chromatographed in adjacent lanes, were visualized by spraying these marker lanes with ninhydrin (Touchstone and Dobbins, 1978). Cold carrier standards were co-chromatographed with the acid 1 fraction and visualized with bromocresol purple and ammonium hydroxide (Touchstone and Dobbins, 1978). In a similar manner, phosphorylated compounds in the acid 2 fractions were identified using ammonium molybdate (Hanes and Isherwood, 1949).

In order to check the accuracy of the procedures outlined previously, in some experiments individual compounds were also separated by 2-dimensional

thin-layer chromatography of samples taken prior to ion-exchange chromatography. The results were in agreement with those from the procedures outlined above with regard to the identity of, and percent ^{14}C incorporated in, individual compounds. Solvent systems used were sec-butanol-formic acid-water (6:1:2), ethanol-ammonium hydroxide-water (6:1:1.6) and the phenol-water and butanol-propionic acid-water system of Pratt and Rand (1979).

Results

For Hydrilla in both the low and high photorespiration states, greater than 50% of the label that was incorporated after 20 s photosynthesis in 20 μM $H^{14}CO_3^-$ was recovered in the C_4 acids malate and aspartate (Fig. 4). During the ensuring chase period in unlabeled CO_2, the low photorespiration Hydrilla plants showed a rapid decline in the percent ^{14}C in both malate and aspartate, after a 1 min lag period (Fig. 4). This decline continued for 60 min. In contrast, in the high photorespiration plants the malate did not decline during the chase, but instead exhibited an increase in percent ^{14}C incorporation (Fig. 4). Unlike malate, the aspartate labeling of high photorespiration plants declined steadily, dropping from 28 to 5% during the 2 h chase (Fig. 4). This decline in aspartate coincided with the increase in malate labeling (Fig. 4), and also with increases in the amino acids glutamate and alanine (data not shown).

The data in Figure 5 demonstrate that for the low photorespiration Hydrilla plants, the decline in malate labeling during the chase period was relatively rapid, with a 50% decrease occurring within 180 s. During this time period, there was a steady and concomitant increase in ^{14}C

Figure 4. Incorporation of ^{14}C into malate and aspartate for Hydrilla in the low and high photorespiration states during a pulse-chase experiment. The zero time points represent the start of the chase following a 20 s pulse. Incorporation is expressed as a percent of the total ^{14}C incorporated. The ^{14}C fixation rates were 12.8 ± 2.2 and 13.4 ± 1.5 μmol CO_2/mg Chl·h for low and high photorespiration Hydrilla, respectively.

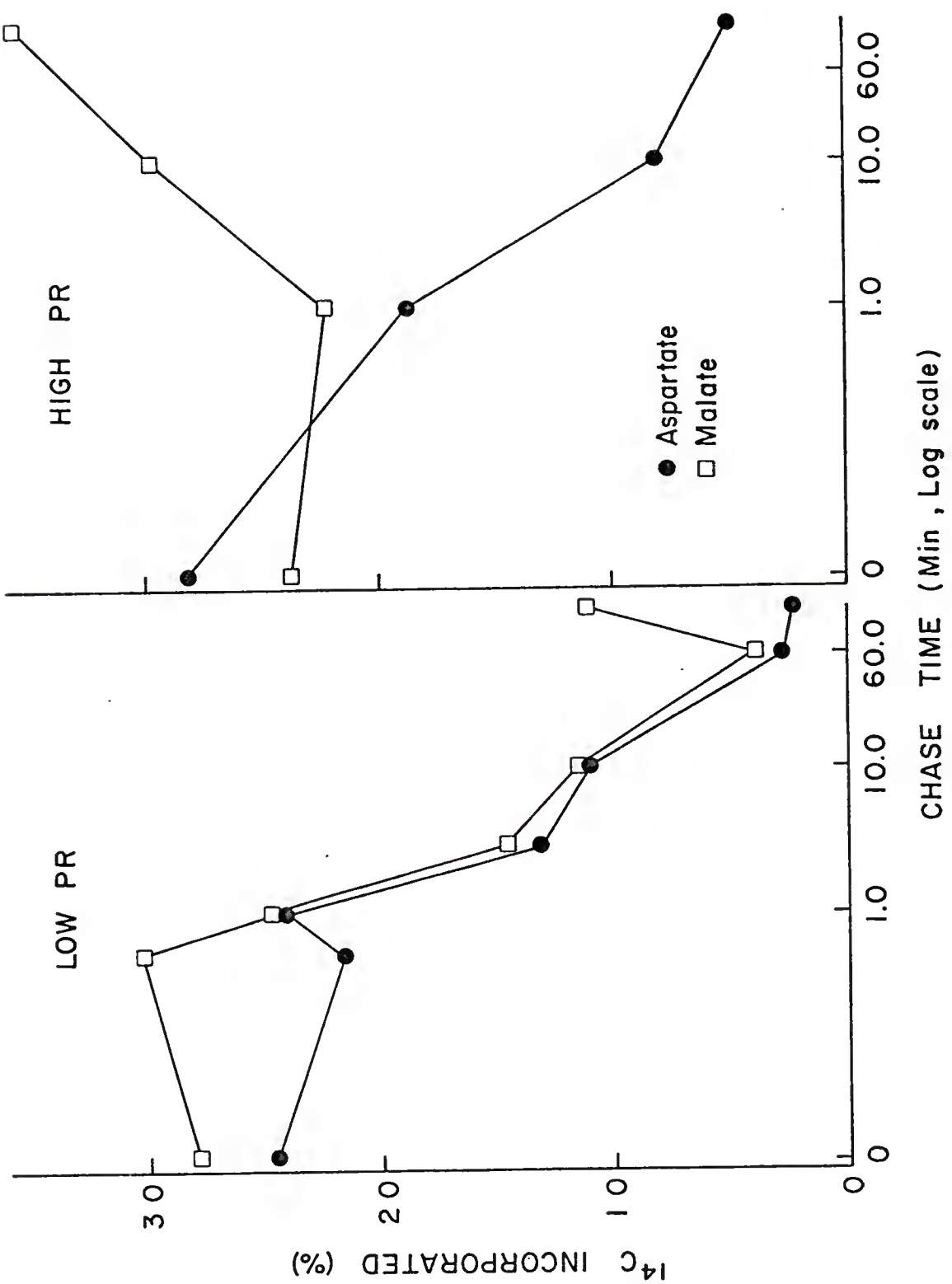
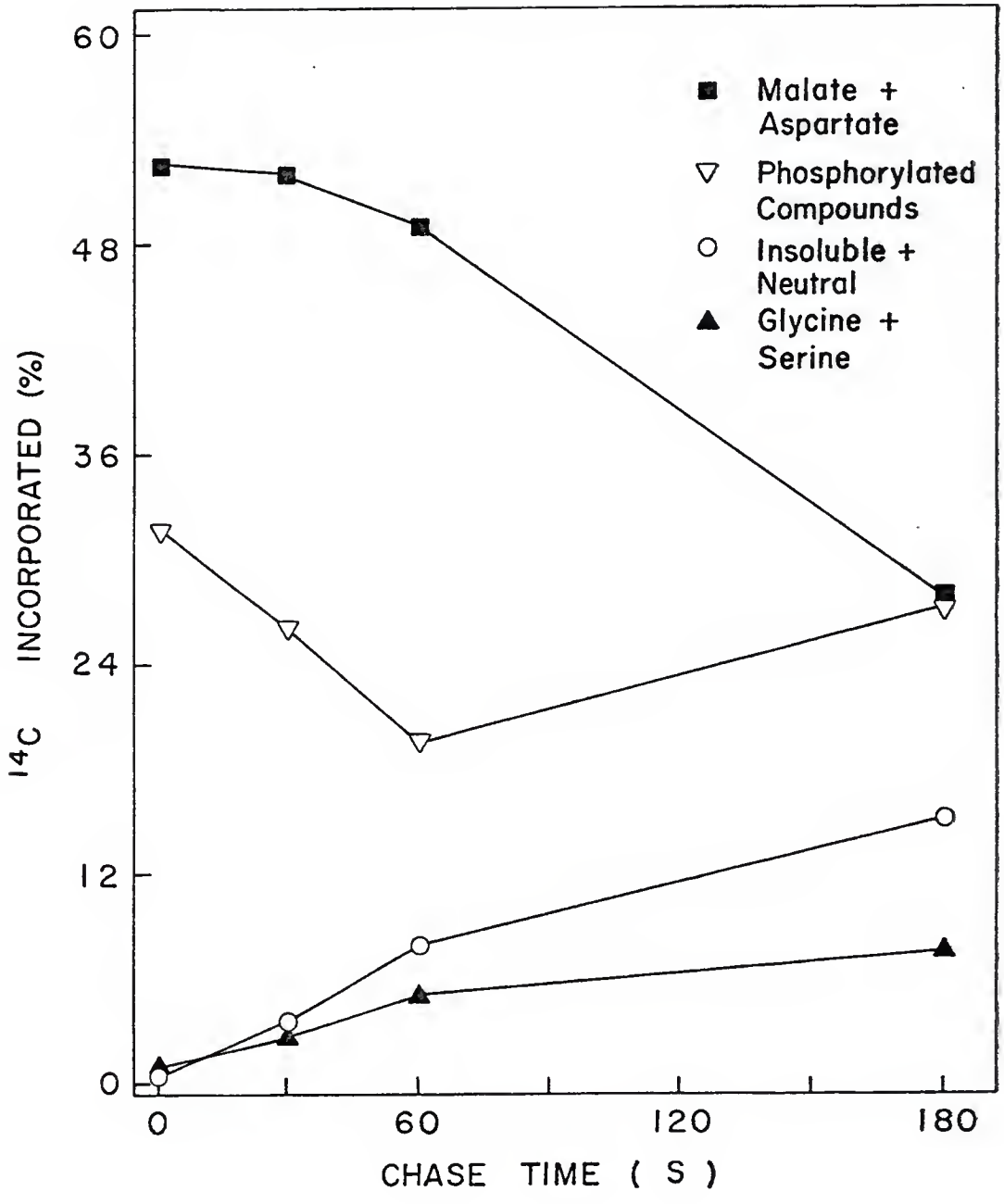

HIGH PR
LOW PR
Aspartate
Malate
0
1.0
10.0
60.0
CHASE TIME (Min , Log scale)
0
10
20
30
^{14}C INCORPORATED (%)

Figure 5. Distribution of ^{14}C among photosynthetic intermediates of Hydrilla in the low photorespiration state during the initial period of the pulse-chase experiment. The zero time point represents the start of the chase following a 20 s pulse. Incorporation is expressed as a percent of the total ^{14}C incorporated and the ^{14}C fixation rates were as in Figure 4.

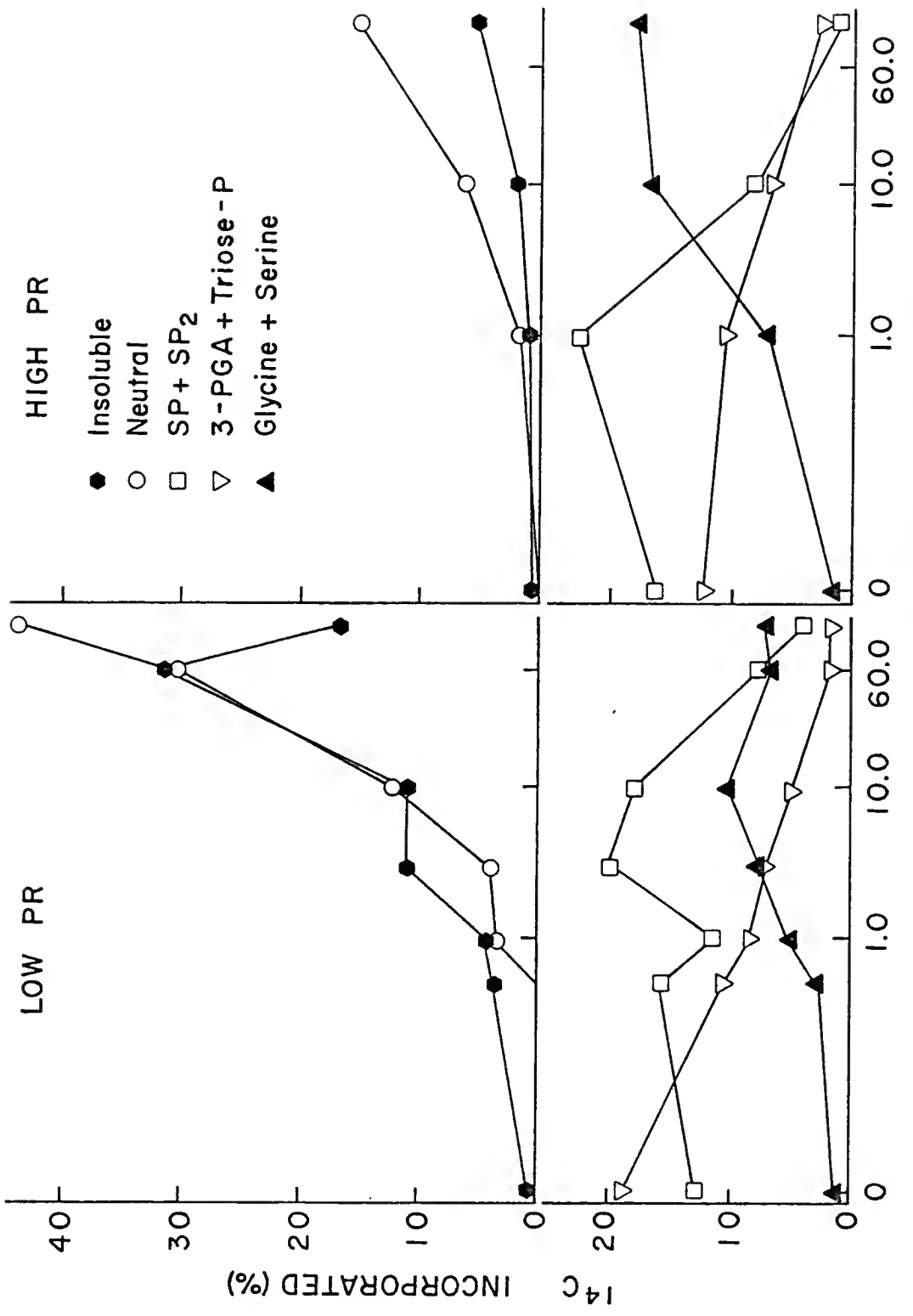

60
48
36
24
12
0
^{14}C INCORPORATED (%)
0
60
120
180
CHASE TIME (S)
Malate + Aspartate
Phosphorylated Compounds
Insoluble + Neutral
Glycine + Serine

entering the neutral and insoluble fractions, composed largely of sucrose and starch, respectively (Fig. 5). Glycine and serine labeling also rose during the chase period. Concomitant with the rise in sucrose and starch, the percent label in total phosphorylated compounds showed a slight tendency to decline from an initial value of 32% (Fig. 5).

Figure 6 shows in more detail the labeling patterns for Hydrilla during the extended 2 h chase period. Both high and low photorespiration plants initially exhibited similar percentages of ^{14}C incorporation into Calvin cycle intermediates. During the chase period, for both photorespiration types, the phosphorylated intermediates gradually decreased as a percentage of the total labeled products, coinciding with an increased labeling of the neutral (sucrose) and insoluble (starch) fractions (Fig. 6). However, after a 2 h chase, neutral and insoluble compounds accounted for more than 60% of the total ^{14}C incorporated by low photorespiration Hydrilla, as compared to less than 23% for the plants in the high photorespiration state (Fig. 6). At all times during the chase, the high photorespiration plants incorporated a greater fraction of the label into the photorespiratory intermediates glycine and serine than did the low photorespiration plants (Fig. 6). For example, after 2 h of chase, glycine and serine comprised 18% of the labeled products of the plants in the high photorespiration state as compared with only 7% in the low photorespiration state (Fig. 6).

For both the high and low phororespiration plants, total dpm incorporated during the pulse showed only a slight decrease during the chase period, even after a 2 h chase. This indicates that ^{14}C loss due to the excretion of labeled compounds such as glycolate must have been negligible, and that the percent changes in the various compounds reflect actual dpm changes.

Figure 6. Distribution of ^{14}C among the photosynthetic intermediates (excluding C_4 acids) of Hydrilla in the low and high photorespiration states during an extended pulse-chase experiment. The zero time points represent the start of the chase following a 20 s pulse. Incorporation is expressed as a percent of the total ^{14}C incorporated and the ^{14}C fixation rates were as in Figure 4.


HIGH PR
LOW PR
Insoluble
Neutral
SP + SP2
3-PGA + Triose-P
Glycine + Serine
14C INCORPORATED (%)
CHASE TIME (Min, Log Scale)
0
10
20
30
40
1.0
10.0
60.0

In contrast to Hydrilla, carbon assimilation in low photorespiration state Myriophyllum was almost exclusively via the Calvin cycle as C_4 acids were less than 10% of the initial labeled products while sugar-P and P-glycerate comprised 77% (Fig. 7). The fraction of ^{14}C incorporated into C_4 acids did not increase at low inorganic carbon concentrations, even when the equilibration and fixation were carried out at only 1 μM HCO_3^- (data not shown). After a 20 s fixation period at 20 μM $H^{14}CO_3^-$, label in phosphorylated compounds decreased steadily during the chase period; this decrease coincided with a substantial increase in labeling of compounds in the insoluble and neutral fractions (Fig. 7).

No major differences in C_4 acid and sugar-P labeling patterns were evident when the low photorespiration Myriophyllum plants were treated with ethoxyzolamide to repress the low photorespiration state (Table 5). However, Myriophyllum plants treated with ethoxyzolamide did exhibit an increased labeling of glycolate, glycine, and serine after a 20 s fixation period (Table 5) and through 10 min of a subsequent chase (data not shown). Low photorespiration Hydrilla plants, in contrast, showed no affect of ethoxyzolamide on the labeling pattern of these photorespiratory intermediates or on the C_4 acids malate and aspartate (Table 5). Sugar monophosphates did increase somewhat, apparently at the expense of P-glycerate and triose-P. For both Hydrilla and Myriophyllum, ethoxyzolamide treatment reduced the total ^{14}C incorporated.

Isonicotinic acid hydrazide, an inhibitor of the glycine to serine conversion in photorespiration, can be used to halt the flow of label through the photorespiratory pathway by inhibiting the further metabolism of glycine (Servaites and Ogren, 1977). When detached leaves of low photorespiration Myriophyllum were treated with increasing concentrations of INH,

Figure 7. Distribution of ^{14}C among the photosynthetic intermediates of Myriophyllum in the low photorespiration state during an extended pulse-chase experiment. The zero time points represent the start of the chase following a 20 s pulse. Incorporation is expressed as a percent of the total ^{14}C incorporated. The ^{14}C fixation rate was 11.0 ± 1.4 µmol CO_2/mg Chl·h.

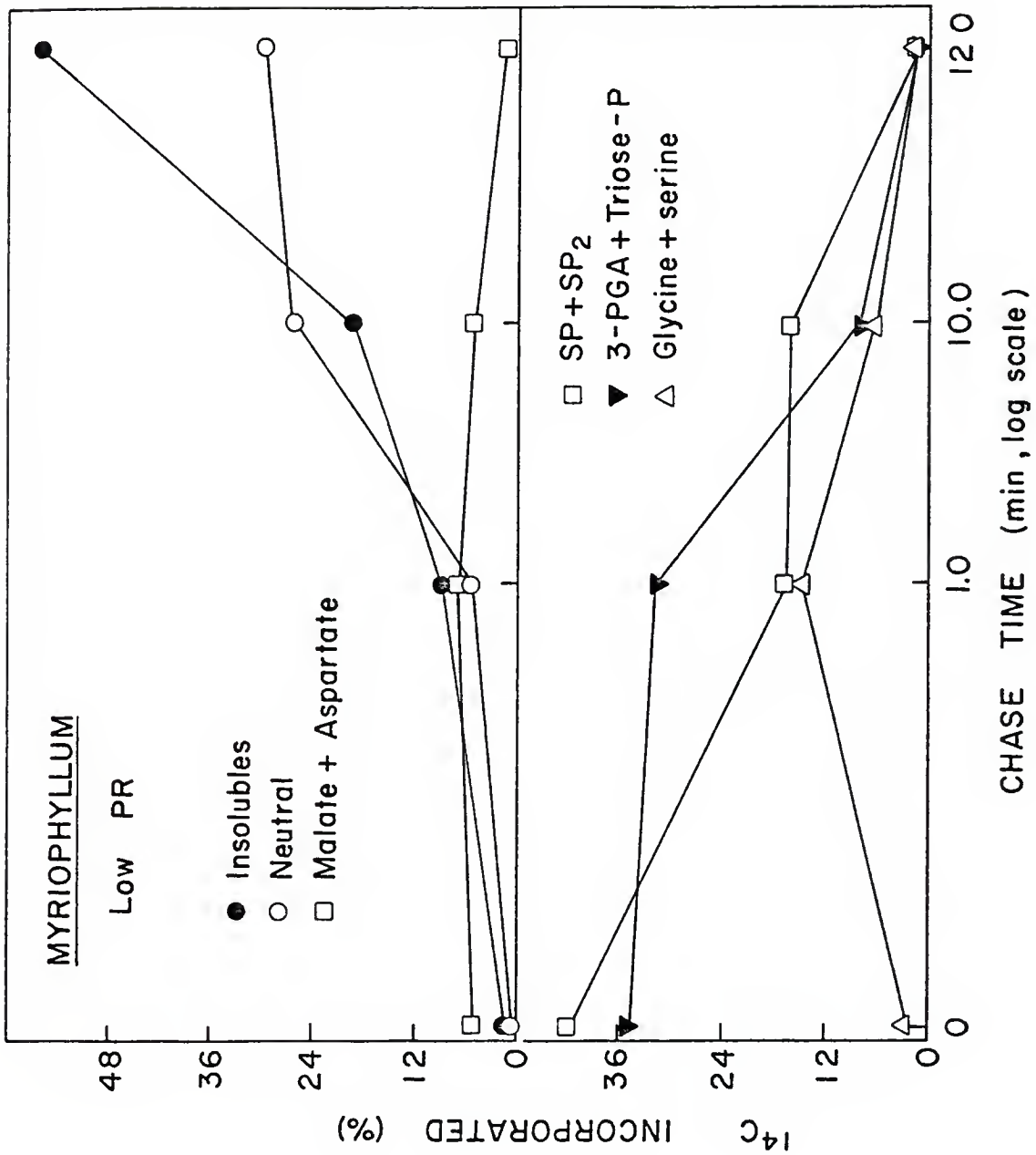

MYRIOPHYLLUM
Low PR
● Insolubles
○ Neutral
□ Malate + Aspartate
□ SP+SP2
▼ 3-PGA + Triose-P
△ Glycine + serine
48
36
24
12
0
36
24
12
0
0
1.0
10.0
120
CHASE TIME (min, log scale)
14C INCORPORATED (%)

Table 5. Effect of ethoxyzolamide (100 µM) on the distribution of ^{14}C among the photosynthetic intermediates of Myriophyllum and Hydrilla in the low photorespiration state.

^{14}C Incorporated	Myriophyllum		Hydrilla	
	Control	Ethoxyzol-amide	Control	Ethoxyzol-amide
Total ^{14}C Fixed *(µmol CO_2/mg Chl·h)*	13.8	10.0	13.6	9.1
Fraction *(% of ^{14}C incorporated)*				
BASIC	6.7	9.2	30.3	23.8
Aspartate	1.4	0.6	24.6	18.8
Alanine	0.4	0.6	1.0	0.7
Glycine + Serine	2.8	7.1	1.0	1.1
ACID 1	7.0	9.8	33.5	41.1
Malate	4.0	4.5	27.9	31.8
Glycolate	1.1	4.8	0.4	0.4
ACID 2	84.1	81.0	35.4	32.2
Sugar-P	35.8	23.2	6.3	14.5
Sugar-P_2	19.1	24.8	6.6	7.3
P-glycerate + Triose-P	27.3	25.2	18.9	5.8
INSOLUBLE	1.8	1.8	0.8	2.8

Plants were pulsed for 20 s at 20 µM $H^{14}CO_3^-$ and 21% O_2 (gas phase).

glycine represented an increasingly larger percentage of the ^{14}C incorporated (Fig. 8). At a saturating concentration of 10 mM INH, 30% of the ^{14}C incorporated by low photorespiration state Myriophyllum was recovered in glycine as compared with less than 15% in the control (Fig. 8). As has been reported for terrestrial plants (Kumarasinghe et al., 1977; Servaites and Ogren, 1977), total ^{14}C fixation decreased in plants treated with 10 mM INH to 70% of the control rate (Fig. 8). Unlike in terrestrial plants, however, serine did not decrease as a percentage of the ^{14}C incorporated. At 5 mM INH, the concentration used in subsequent experiments, excretion of organic ^{14}C by Myriophyllum was less than 3% of the total ^{14}C fixed, which was the same rate as the control (data not shown).

For Myriophyllum in both the high and the low photorespiration state, 5 mM INH caused label to accumulate in glycine, apparently at the expense of neutral and acid 2 compounds (Table 6). In the presence of INH, glycine represented a much greater proportion of the photosynthetically labeled products in high than in low photorespiration plants; the percent values were 48 and 25, respectively (Table 6). In the absence of INH, little difference in glycine formation between the two photorespiratory states was evident (Table 6). The INH had a similar effect on ^{14}C incorporation into glycine in Hydrilla (Table 6).

When glycine accumulation was examined at 20 μM HCO_3^- with respect to the O_2 concentration, at O_2 concentrations greater than 1 or 2% (gas phase), glycine comprised a greater fraction of the ^{14}C labeled products in the high than in the low photorespiration state of Myriophyllum (Fig. 9). At 80% O_2, glycine constituted only 25% of the total ^{14}C incorporated by the low photorespiration plants compared with greater than 45% in the high photorespiration state (Fig. 9). In both photorespiration states, formation

Figure 8. The effect of isonicotinic acid hydrazide (INH) concentration on total ^{14}C fixation and the incorporation of ^{14}C into glycine and serine for Myriophyllum in the low photorespiration state at 20 μM $H^{14}CO_3^-$ and 50% O_2 (gas phase). Incorporation into glycine and serine at each INH concentration is expressed as a percent of the total ^{14}C fixed in 15 min at that INH concentration. The 100% (control) ^{14}C fixation rate without INH was 11.3 μmol CO_2/mg Chl·h.

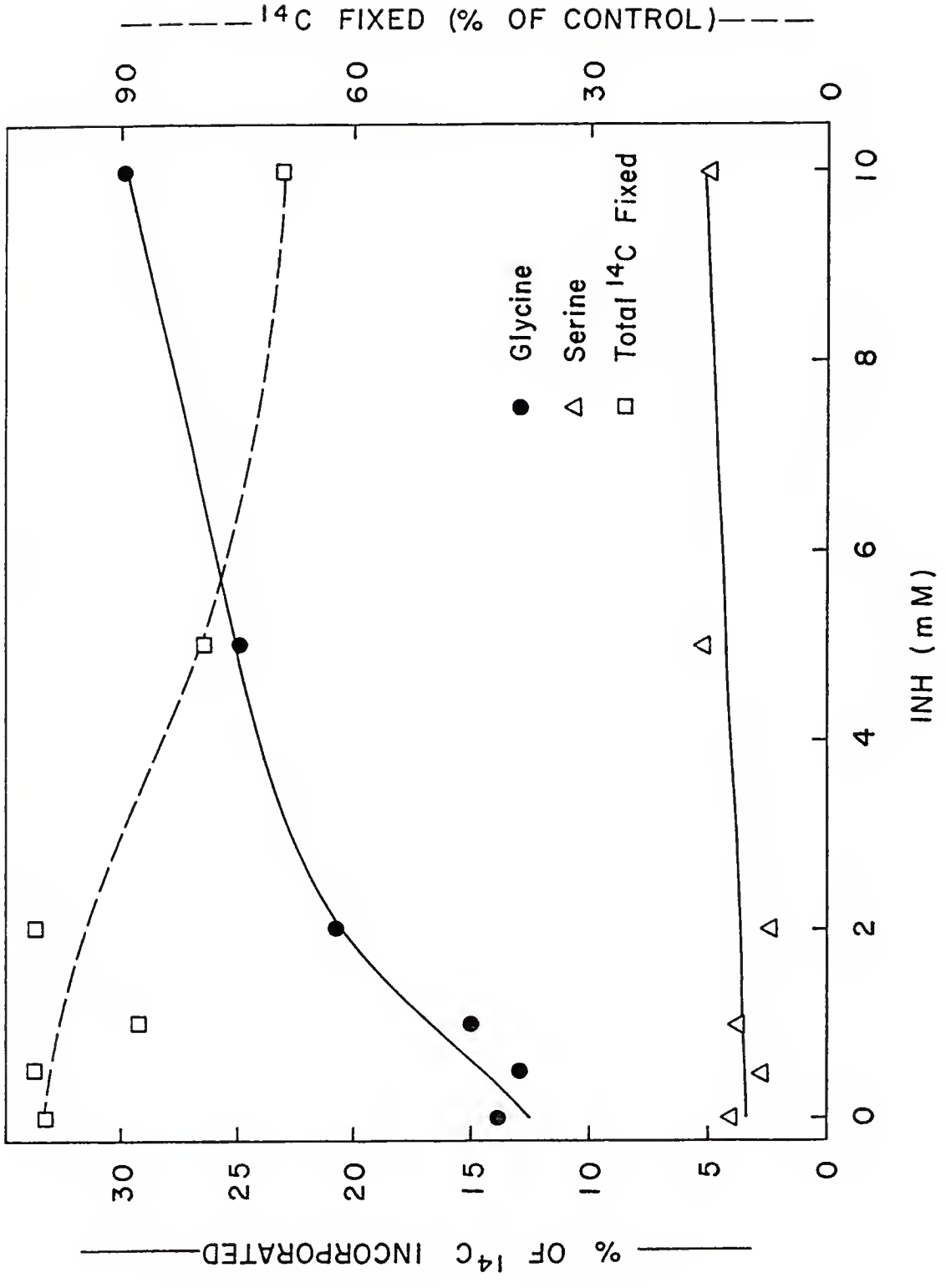
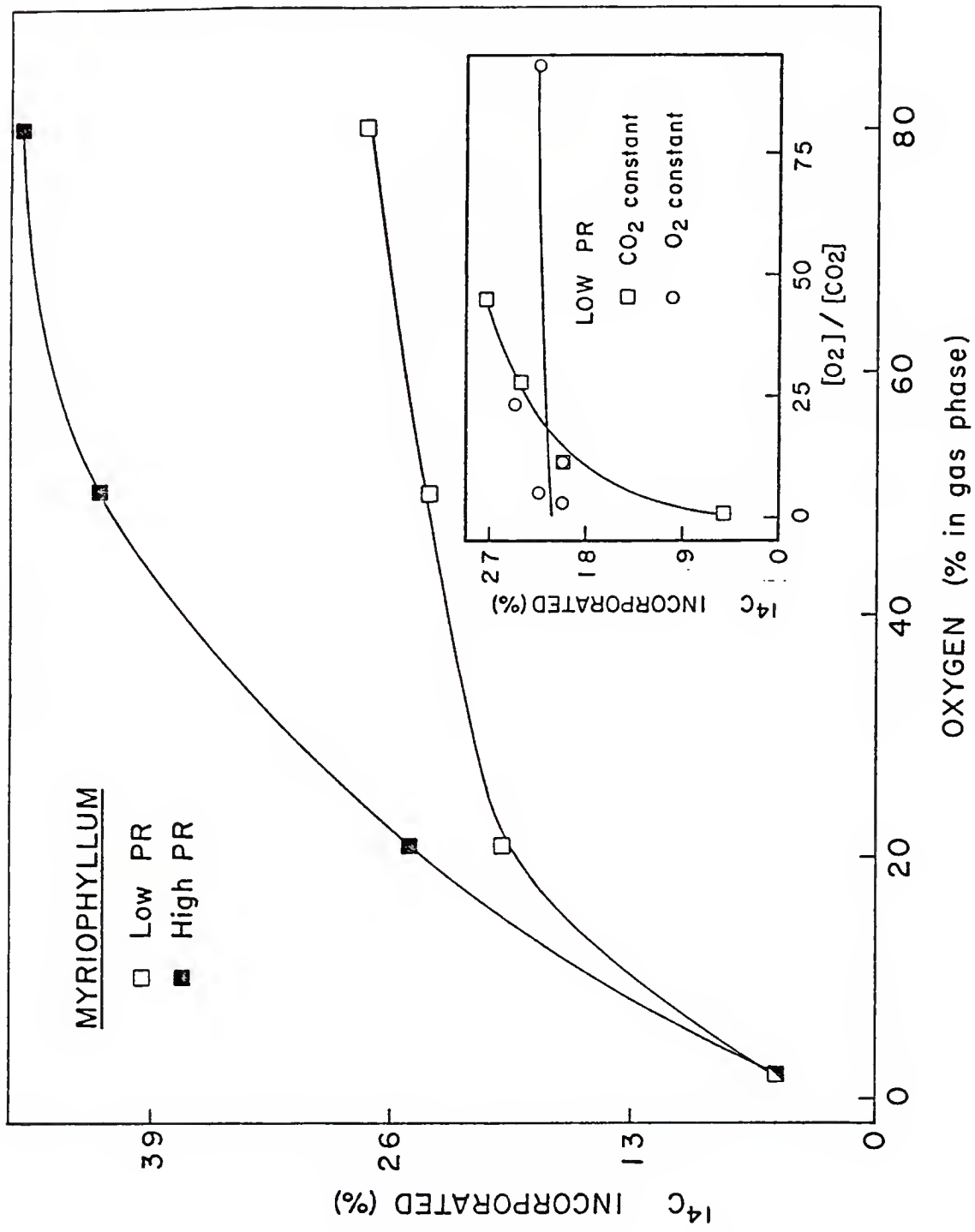

——— % OF ^{14}C INCORPORATED ———
30
25
20
15
10
5
0
90
60
30
0
——— ^{14}C FIXED (% OF CONTROL) ———
Glycine
Serine
Total ^{14}C Fixed
0
2
4
6
8
10
INH (mM)

Table 6. The effect of isonicotinic acid hydrazide (5 mM INH) on the distribution of ^{14}C among the photosynthetic intermediates of Myriophyllum and Hydrilla in the low and high photorespiration (PR) states.

^{14}C Incorporated	Myriophyllum				Hydrilla			
	Low PR		High PR		Low PR		High PR	
	Control	INH	Control	INH	Control	INH	Control	INH
Total ^{14}C Fixed *(μmol CO_2/mg Chl·h)*	11.3	9.0	5.4	3.3	4.2	2.9	3.5	2.9
Fraction *(% of ^{14}C incorporated)*								
BASIC	34.4	48.7	23.8	77.2	23.2	33.2	33.2	58.7
Aspartate	4.8	5.4	0.7	7.6	5.8	10.0	11.6	26.1
Glycine	14.0	25.4	11.0	47.5	5.8	9.5	6.1	13.7
Serine	4.2	7.9	3.6	10.0	1.6	1.8	4.6	2.3
NEUTRAL	25.6	19.5	11.8	5.7	21.2	11.2	5.8	4.0
ACID 1	8.8	5.0	13.2	2.3	25.5	25.8	42.5	26.1
ACID 2	29.3	16.9	49.1	12.5	24.4	20.1	16.6	10.4
INSOLUBLE	1.9	9.7	2.6	2.3	4.4	7.6	1.7	1.4

Plants were exposed to ^{14}C in the light for 15 min at 20 μM $H^{14}CO_3^-$ and 50% O_2 (gas phase).

Figure 9. The effect of O_2 concentration on the incorporation of ^{14}C into glycine for Myriophyllum in the low and high photorespiration states during 15 min photosynthesis at 20 μM $H^{14}CO_3^-$ and 5 mM INH. Incorporation is expressed as a percent of the total ^{14}C incorporated at each O_2 concentration.
Inset. The effect of the external O_2/CO_2 concentration ratio on the incorporation of ^{14}C into glycine for Myriophyllum plants in the low photorespiration states during photosynthesis in 5 mM INH. Experiments were performed either at a constant $H^{14}CO_3^-$ concentration of 20 μM or at a constant O_2 concentration of 21% (gas phase) and HCO_3^- concentrations ranging from 2.5 to 100 μM.


MYRIOPHYLLUM
Low PR
High PR
^{14}C INCORPORATED (%)
0
13
26
39
0
20
40
60
80
OXYGEN (% in gas phase)
LOW PR
CO_2 constant
O_2 constant
^{14}C INCORPORATED (%)
0
9
18
27
0
25
50
75
[O2] / [CO2]

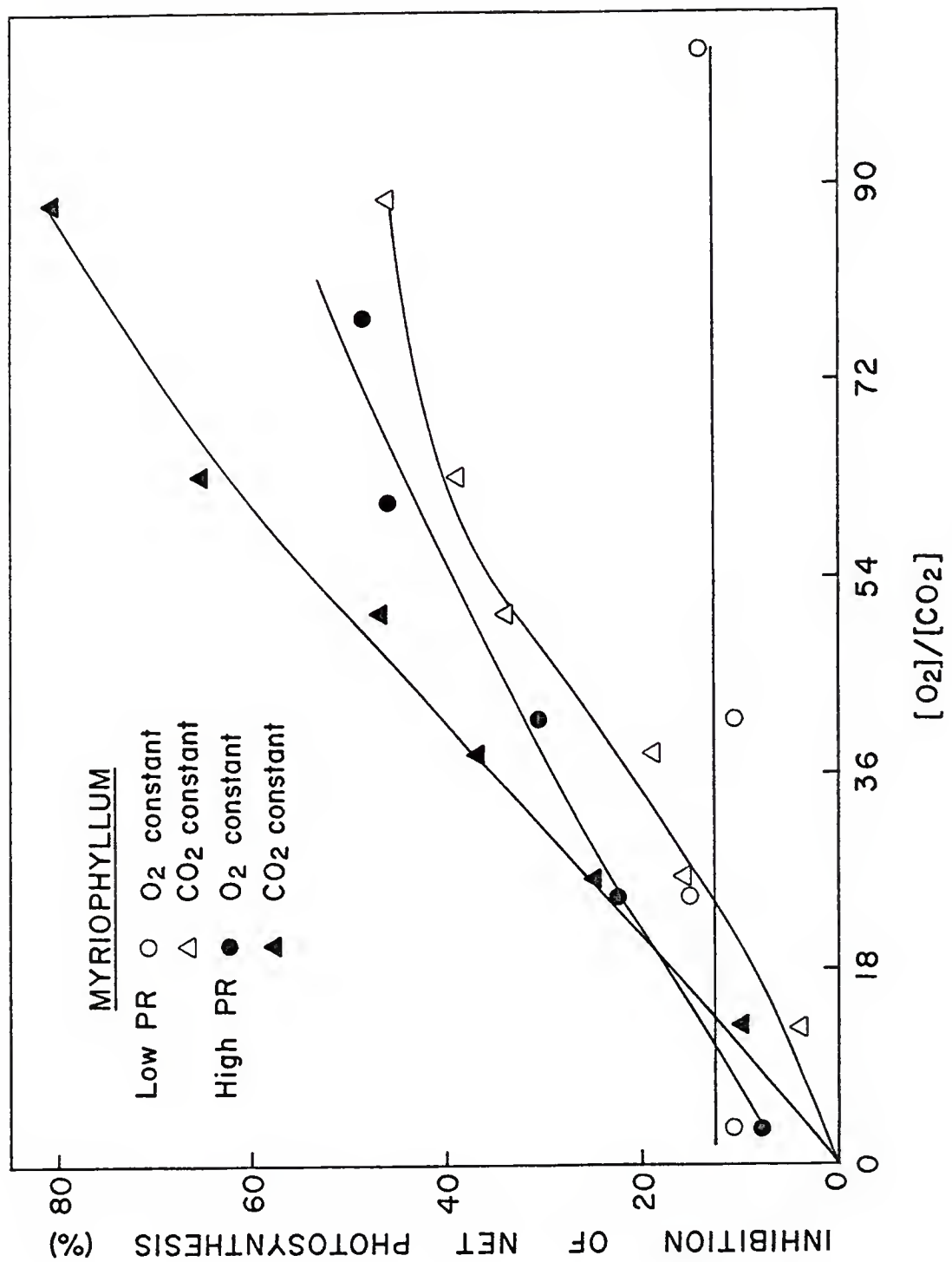
of labeled glycine at 20 μM HCO_3^- approached saturation at O_2 concentrations greater than 50%. When measured under a constant O_2 concentration, glycine labeling in low photorespiration Myriophyllum was independent of the HCO_3^- concentration and, therefore, was not a function of the O_2/CO_2 ratio (Fig. 9, inset).

A similar response pattern to that observed for glycine labeling was recorded for O_2 inhibition of net photosynthesis in low but not high photorespiration state plants. Thus, for plants in the low photorespiration state, the percent inhibition of net photosynthesis by 21% O_2 was independent of the CO_2 concentration and therefore inhibition of net photosynthesis by O_2 was not a function of the external O_2/CO_2 ratio (Fig. 10). However, inhibition of net photosynthesis was affected by the O_2 concentration when measured under a constant CO_2 concentration (330 μl CO_2/l). In contrast to these results with low photorespiration plants under either constant O_2 or constant CO_2, the percent inhibition of net photosynthesis by O_2 in high photorespiration state Myriophyllum responded to changes in both O_2 and CO_2 and was thus a function of the external O_2/CO_2 ratio (Fig. 10). The percent inhibition of net photosynthesis by O_2 was considerably less in the low photorespiration plants than in the high photorespiration plants whether measured under conditions of either constant O_2 or constant CO_2 (Fig. 10).

Discussion

Evidence for C_4 acid metabolism has been presented for a number of submersed angiosperm species based either on direct data from labeling studies (Brown et al., 1974; De Groote and Kennedy, 1977; Browse et al., 1980; Holaday and Bowes, 1980; Beer and Wetzel, 1982; Helder and van Harmelen,

Figure 10. The effect of the external O_2/CO_2 concentration ratio on the percent inhibition of net photosynthesis by O_2 for Myriophyllum plants in the low and high photorespiration states. Inhibition was measured as a decrease from the net photosynthetic rate at 1% O_2 (gas phase). Infrared gas analysis measurements were made either at 327 µl CO_2/l and from 1% to 80% O_2 (constant CO_2), or 21% O_2 and from 50 to 2,500 µl CO_2/l (constant O_2) gas concentrations.


MYRIOPHYLLUM
Low PR ○ O2 constant
△ CO2 constant
High PR ● O2 constant
▲ CO2 constant
INHIBITION OF NET PHOTOSYNTHESIS (%)
0
20
40
60
80
0
18
36
54
72
90
[O2]/[CO2]

1982) or indirectly by implication from measurements of relatively high PEP carboxylase activities (Bowes et al., 1978; Salvucci and Bowes, 1981). All of the angiosperms cited are monocotyledonous and five genera, Egeria, Elodea, Hydrilla, Lagarosiphon, and Valisneria, belong to one family, Hydrocharitaceae. Labeling studies with Hydrilla and Egeria have shown that the proportion of label incorporated into C_4 acids in the light increases as the level of inorganic carbon is decreased (Browse et al., 1979a; Holaday and Bowes, 1980). Thus the formation of C_4 acids in photosynthesis by certain submersed angiosperm species may be an adaptation to low external levels of inorganic carbon (Van et al., 1976) and appears to be associated with the low photorespiration state which is induced under these conditions (Holaday and Bowes, 1980; Salvucci and Bowes, 1981).

In Hydrilla, although C_4 acids accounted for a similarly large percentage of the photosynthetically fixed carbon in both the low and the high photorespiration states, differences in the fate of malate were apparent between the respective photorespiration states. In the low photorespiration state plants, as in terrestrial C_4 plants, malate was utilized to form photosynthetic end-products (sucrose and starch), probably as a result of the activities of C_4-pathway enzymes which increase with induction of the low photorespiration state (Salvucci and Bowes, 1981). This fixation and decarboxylation of C_4 acids in the light and the enzymatic capacity to regenerate PEP via pyruvate Pi dikinase are features similar to the photosynthetic carbon metabolism in terrestrial C_4 plants (Chollet and Ogren, 1975), and in an analogous manner they may also function as a CO_2-concentrating mechanism in low photorespiration Hydrilla. The resulting increase in the concentration of CO_2 at the site

of RuBP carboxylase-oxygenase could explain the C_4-like photosynthesis/photorespiration ratio which is characteristic of Hydrilla in the low photorespiration state (Bowes et al., 1978; Salvucci and Bowes, 1981; Holaday et al., in press).

In contrast, in the high photorespiration state of Hydrilla, label was accumulated in malate throughout an extended chase period and did not show any evidence of turnover. This lack of malate turnover during photosynthesis is probably the result of the low activities of C_4 enzymes in the high photorespiration plants (Salvucci and Bowes, 1981). It is nonetheless unusual, considering the large percentage of carbon that was incorporated into this compound. Similar results have been reported for Egeria plants with high CO_2 compensation points (Browse et al., 1980). In these Egeria plants, the malate pool expands during the light period and decreases in the dark, which suggests that malate formed in the high photorespiration state is an end-product of photosynthesis, possibly being used for some function such as ion (HCO_3^-) balance rather than as a source of CO_2 for RuBP carboxylase-oxygenase (Browse et al., 1980). In the low photorespiration state, the available evidence with Hydrilla indicates that the malate pool fluctuates with an opposite diel rhythm, as higher levels of titratable acidity are measured after a dark than after a light period (Holaday and Bowes, 1980). No measurements of malate pool sizes were made in the present study, but it is likely that in the low photorespiration state, under natural conditions, the malate pool increases during the dark period due to the relatively high capacity for dark fixation in the low photorespiration plants (Holaday and Bowes, 1980) and then decreases in the light as malate is utilized in photosynthesis.

This scheme for malate metabolism in low, but not in high, photorespiration plants is somewhat reminiscent of terrestrial CAM plants, and diurnal acid patterns similar to that reported by Holaday and Bowes (1980) for Hydrilla have since been reported for the submersed lower vascular plant Isoetes (Keeley, 1981) and the submersed angiosperm Scirpus (Beer and Wetzel, 1981). Unlike the classical CAM situation, however, it appears that in Hydrilla some fixation into malate potentially can occur in the light, as well as in the dark.

To date, at least three genera of aquatic angiosperms (Myriophyllum, Ceratophyllum, and Ranunculus), all dicotyledonous, are known to lack significant C_4 acid metabolism based either on C_3-type labeling patterns (Stanley and Naylor, 1972) or on low levels of PEP carboxylase activity (Van et al., 1976; Salvucci and Bowes, 1981; Valanne et al., 1982). The pulse-chase labeling data presented here confirm the C_3-type labeling patterns for Myriophyllum. Two of the three groups, Myriophyllum and Ceratophyllum, are known to exhibit variable photorespiration states (Salvucci and Bowes, 1981; Holaday et al., 1982) like Hydrilla, despite the lack of a C_4 acid-type mechanism to achieve the low photorespiration state. In this regard they seem to be similar photosynthetically to low CO_2-grown unicellular green algae (Berry et al., 1976; Beardall and Raven, 1981). As with the algae the mechanism involved in reducing photorespiration in these submersed angiosperms appears to depend, in part, on the activity of carbonic anhydrase as demonstrated by the manner in which the carbonic anhydrase inhibitor ethoxyzolamide altered the gas exchange characteristics (CHAPTER II) and increased the labeling of photorespiratory intermediates in low photorespiration Myriophyllum plants (Table 5).

Low CO_2-grown algae probably utilize an inorganic carbon accumulating system in conjunction with the carbonic anhydrase activity, in order to concentrate CO_2 at the active site of fixation by RuBP carboxylase-oxygenase (Badger et al., 1978; Spalding et al., 1982). The labeling data in the present study are consistent with the operation of a similar system in Myriophyllum in the low photorespiration state. Firstly, the increase in glycine labeling which occurred in the presence of INH suggests that the low photorespiration state in Myriophyllum is not the result of an alternate pathway that bypasses the reactions involved in the release of photorespiratory CO_2. Secondly, the reduced label in glycine of low, as compared to high, photorespiration plants suggests that less carbon passes through the photorespiratory pathway, as opposed to there being an increase in refixation of photorespired CO_2 in the low photorespiration plants. Both of these lines of evidence support the contention that the reduction of photorespiratory CO_2 release in low photorespiration Myriophyllum is due to elevated CO_2 at the site of RuBP carboxylase-oxygenase. Similar arguments can be made for low photorespiration Hydrilla from the inhibitor and labeling data presented in this study (Fig. 6 and Table 6).

When evaluated as a function of changing O_2 but constant inorganic carbon concentrations, at any given $[O_2]/[CO_2]$ ratio, the percentage of ^{14}C incorporated into glycine in high photorespiration state Myriophyllum was virtually identical to the percentage reported for leaf cells isolated from the C_3 plant, soybean, treated similarly with INH (Servaites and Ogren, 1977). In contrast, glycine labeling in low photorespiration Myriophyllum was considerably less than that reported for soybean cells, but responded in a similar manner to O_2 by increasing with increasing O_2 concentration. From the results of the reverse experiment, in which the

O_2 was held constant and the inorganic carbon level was varied, it appears that at a given O_2 concentration the percent of glycine labeling and the percent of O_2 inhibition of net photosynthesis in low photorespiration state Myriophyllum, by being responsive only to changes in the O_2 concentration, are not strictly a function of the $[O_2]/[CO_2]$ ratio. Shelp and Canvin (1980) have reported a similar response in low CO_2-grown Chlorella cells. These authors suggested that the O_2 inhibition of net photosynthesis exhibited by Chlorella may not be photorespiratory in nature (Shelp and Canvin, 1980). However, this cannot be the case for Myriophyllum, because when the O_2 concentration was raised, the increased percent of O_2 inhibition of net photosynthesis was accompanied by an increase in the percent labeling of the photorespiratory intermediate glycine.

These gas exchange and labeling characteristics exhibited by Myriophyllum plants in the low photorespiration state, in response to changes in the external CO_2 and O_2, superficially appear to be inconsistent with the properties of RuBP carboxylase-oxygenase. In vitro determinations of the v_o/v_c ratio of the enzyme from low photorespiration state Myriophyllum using several CO_2 and O_2 concentrations indicated that the relative activity of the carboxylase and oxygenase is a linear function of the $[O_2]/[CO_2]$ ratio as is the case for RuBP carboxylase-oxygenase from other sources (Jordan and Ogren, 1981b) including the high photorespiration state Myriophyllum (APPENDIX A). Thus, for Myriophyllum in the low photorespiration state, the observed physiological responses of photosynthesis and photorespiration, which are catalyzed by RuBP carboxylase and oxygenase, respectively, are probably not due to any kinetic changes in the two activities of this enzyme, but rather appear due to an uncoupling of the enzyme from the external $[O_2]/[CO_2]$ ratio. Since the

diffusion of CO_2 and O_2 into the active site of the RuBP carboxylase-oxygenase is one of the factors determining the rates of photosynthesis and photorespiration, it may be possible that carbonic anhydrase, in association with a HCO_3^- accumulation system, modulates the internal pool of inorganic carbon that exists in both the HCO_3^- and free CO_2 form so as to act as a "buffer" for CO_2. This could, to some extent, uncouple CO_2 at the site of fixation from the external CO_2 concentration without affecting the access of external O_2 to the oxygenation site. The observed physiological responses could then still be attributed to RuBP carboxylase-oxygenase but in association with the accumulation/utilization mechanism dependent on carbonic anhydrase.

Under certain conditions, carbonic anhydrase has been shown to enhance CO_2 fixation by isolated RuBP carboxylase and this enhancement (as a percent) increases with decreasing CO_2 concentration (Bird et al., 1980, 1982). Tsuzuki et al. (1980) have observed a similar enhancement effect of photosynthetic CO_2 fixation by adding carbonic anhydrase exogenously to Chlorella cells. These investigators have postulated that internally, carbonic anhydrase facilitates the utilization of an elevated HCO_3^- pool in the chloroplasts as an indirect source of CO_2 which can enhance photosynthesis to the detriment of photorespiration (Tsuzuki et al., 1980). Carbonic anhydrase may function in a somewhat similar way in the low photorespiration state of submersed angiosperms as evidenced by the enhanced percent of inhibition of net photosynthesis caused by ethoxyzolamide at low CO_2 concentrations for Myriophyllum and Hydrilla in the low, but not the high, photorespiration state (CHAPTER II).

Thus, it appears likely that both C_4 acid- and non-C_4 acid-dependent systems for concentrating CO_2 occur among submersed aquatic angiosperms,

which, in conjunction with carbonic anhydrase, reduce the activity of the photorespiratory carbon oxidation cycle. As with other autotrophic organisms, both systems eventually depend on the operation of the biochemical C_3 carbon reduction cycle for the formation of carbohydrates. However, for C_4 plants, unicellular green algae, or cyanobacteria, possession of the carbon reduction cycle is not in itself a sufficient basis to categorize these organisms as C_3 plants. A similar argument can be made for submersed aquatic macrophytes as their variable photorespiratory capacity and the associated biochemical modifications support the contention that submersed aquatic macrophytes cannot be categorized within any of the photosynthetic groups currently recognized.

APPENDIX A
CHARACTERISTICS OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE-OXYGENASE FROM MYRIOPHYLLUM AND HYDRILLA

Introduction

The substrate specificity factor of RuBP carboxylase-oxygenase (V_cK_o/V_oK_c) can be used to determine the relative rates of the carboxylase and the oxygenase reactions at a given O_2 and CO_2 level (Jordan and Ogren, 1981b). This parameter is an indicator of the balance between the RuBP carboxylation and oxygenation reactions and it has been shown that values for the specificity factor tend to increase with evolution from 47-48 in cyanobacteria, through 54-63 in green algae, to 77-82 in higher plants (Jordan and Ogren, 1981b). A change in the specificity of RuBP carboxylase-oxygenase for CO_2 or O_2 is one possible mechanism that would account for the variable photorespiratory activity in submersed aquatic angiosperms. It was the purpose of this investigation to examine several properties of RuBP carboxylase-oxygenase in submersed angiosperms to determine whether an alteration in the enzyme is directly involved in the photorespiration-reducing mechanisms of Myriophyllum and Hydrilla.

Materials and Methods

Preparation of ^{3}H-RuBP and P-Glycolate Phosphatase

Tritium-labeled RuBP (^{3}H-1-RuBP) was prepared from ^{3}H-2-glucose as described by Jordan and Ogren (1981a). The preparation was 93.5% pure

as judged by ion-exchange and thin-layer chromatography. The specific activity of ^{3}H-RuBP was determined by carboxylation with $NaH^{14}CO_3$ of known specific activity using thrice-crystallized tobacco RuBP carboxylase-oxygenase (Kung et al., 1980). Phosphoglycolate-specific phosphatase was isolated from tobacco leaves as described previously (Jordan and Ogren, 1981a).

Specificity Factor Determinations

Substrate specificity factors were determined for desalted extracts of the aquatic plants and for crystalline tobacco RuBP carboxylase-oxygenase. Plant material was ground at 4°C in 5 ml of 100 mM Bicine, 7.5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM DTT and 1.5% PVP-40, at pH 8.0. The extract was centrifuged for 10 min at 10,000 g, after which the supernatant was treated on a 1.7 x 24 cm column of Sephadex G-25 at 4°C. The column was equilibrated and eluted with CO_2-free buffer; 50 mM Bicine, 10 mM $MgCl_2$, 0.1 mM EDTA, and 5 mM DTT at pH 8.0. Tobacco enzyme was dissolved in the CO_2-free buffer containing 0.1 M NaCl.

The RuBP carboxylase-oxygenase reactions were run for 10 min at 30°C in 5 ml reaction vials containing a final volume of 0.5 ml of 50 mM Bicine, 10 mM $MgCl_2$, 0.1 mM EDTA, 10 mM DTT, $NaH^{14}CO_3$ (2.2 µCi/µmol) and 28 µM ^{3}H-1-RuBP (31.0 µCi/µmol), at pH 8.02. Prior to the addition of CO_2-free Bicine, the vials were purged with CO_2-free N_2/O_2 mixtures. Purging was continued as CO_2-free Bicine, and then 0.05 ml of the plant extracts were added. After 5 min the vials were sealed and, following the addition of $NaH^{14}CO_3$, the reactions were initiated with 10 µl containing 14 nmol of ^{3}H-RuBP.

Assays were run in duplicate and zero time and N_2 purged vials were used as controls. The reaction products were separated and quantified as

described by Jordan and Ogren (1981a), and the specificity factors were calculated from the slope of the linear plot of oxygenase/carboxylase activities (v_o/v_c) versus $[O_2]/[CO_2]$ (Jordan and Ogren, 1981b).

Km Determinations

Plant material from the aquatic plants and tobacco leaves was ground, centrifuged and desalted as described above. The extraction buffer contained 100 mM Tris-HCl, 5 mM DTT, 7.5 mM $MgCl_2$, 0.1 mM EDTA and 1.5% PVP-40 at pH 8.5, and the CO_2-free column buffer was 25 mM Tris-HCl, 5 mM DTT, and 10 mM $MgCl_2$, at pH 8.0. Immediately after elution from the column, the plant extract was collected in a N_2-purged flask, supplemented with $NaH^{14}CO_3$ (2.2 μCi/μmol) to a final concentration of 10 mM and incubated at room temperature for 10 min to activate the enzyme.

Assays were run at 30°C in 5 ml reaction vials containing 0.5 ml of 100 mM Bicine, 5 mM DTT, 10 mM $MgCl_2$, 0.5 mM RuBP, and $NaH^{14}CO_3$ (2.2 μCi/μmol), at pH 7.85. Vials were purged with N_2 or O_2 prior to the addition of CO_2-free Bicine. Vials were sealed and $NaH^{14}CO_3$ was added through a serum stopper. The reactions were initiated with 0.05 ml of plant extract and terminated after 45 s with 3 N HCl. Acid stable dpm were determined as described previously (Van et al., 1976). Km values were determined by the statistical method of Wilkinson (1961) and $Ki(O_2)$ values were calculated from the apparent $Km(CO_2)$ values obtained at 100% O_2.

Rocket Immunoelectrophoresis

Rocket immunoelectrophoresis was performed according to the method of Laurell (1972). Immunoelectrophoresis plates were prepared by pouring 12 ml of a 1% (w/v) solution of melted agarose containing 30 mM barbital,

2 mM calcium lactate, and 0.02% NaN_3, pH 8.6, onto an 8 x 10 cm glass plate. Immediately prior to pouring, 1.0% (v/v) of anti-tobacco RuBP carboxylase-oxygenase IgG was added to the agarose solution. Plant extracts were prepared as described by Rejda et al. (1980) and 2 µl of each sample (≤2 µg total protein) was added to 2 mm wells punched into the plate. Electrophoresis was run at 100 volts/cm for 16 h at 15°C. The electrophoresis buffer was 30 mM barbital, 2 mM calcium lactate, and 0.02% (w/v) NaN_3 at pH 8.6.

The RuBP carboxylase-oxygenase protein content was estimated from peak heights of the samples as compared with the peak heights obtained using known amounts of crystalline tobacco RuBP carboxylase-oxygenase as the standard. Chlorophyll content was determined as described by Arnon (1949) and soluble protein was determined by the method of Spector (1978).

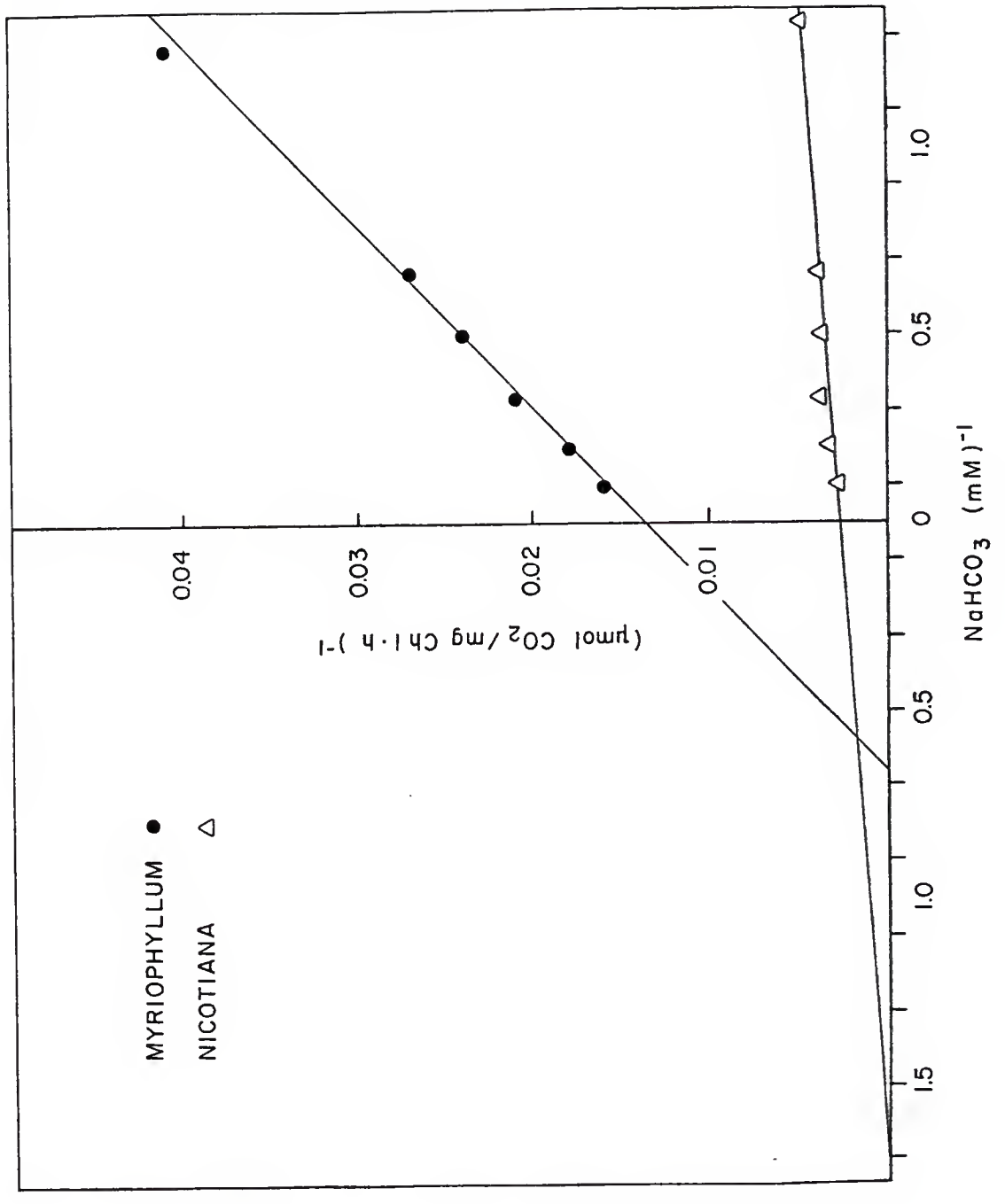
Results and Discussion

The $Km(CO_2)$ values for RuBP carboxylase-oxygenase from Myriophyllum and Hydrilla were similar and the $Km(CO_2)$ and $Ki(O_2)$ values, as well as the Vmax, were not affected by changes in the photorespiration state of the plants (Table 7). The $Km(CO_2)$ values were higher than those for the tobacco enzyme (Table 7, Fig. 11), but similar to the $Km(CO_2)$ values reported for RuBP carboxylase-oxygenase from C_4 plants and green algae (Jordan and Ogren, 1981b). Similarly high $Km(CO_2)$ values have been reported for the enzyme from other submersed macrophyte species (Yeoh et al., 1981). Measurements of the specificity factor indicated that the relative activities of the carboxylase and the oxygenase were similar in the high and low photorespiration states of Myriophyllum (Table 7). Thus,

Table 7. Kinetic parameters of RuBP carboxylase-oxygenase from Myriophyllum and Hydrilla in the low and high photorespiration (PR) states, and from Nicotiana.

Plant and PR state	Km(HCO_3^-) *(mM)*	Km(CO_2) *(uM)*	Ki(O_2) *(mM)*	Specificity factor *(V_cK_o/V_oK_c)*	Vmax *(umol/mg protein·h)*
Myriophyllum					
Low PR	0.71	25.0	0.312	66.2	5.7
High PR	0.74	26.1	0.487	68.4	7.7
Hydrilla					
Low PR	0.79	27.8	0.734	73.2	4.2
High PR	0.73	25.8	0.690	----	4.9
Nicotiana	0.29	10.4	----	80.0	14.9

Figure 11. Double reciprocal plots of RuBP carboxylase activity versus $NaHCO_3$ concentration for high photorespiration (PR) state Myriophyllum and Nicotiana (tobacco).


MYRIOPHYLLUM
NICOTIANA
(μmol CO2/mg Chl·h)-1
0.04
0.03
0.02
0.01
0
0.5
1.0
1.5
0.5
1.0
NaHCO3 (mM)-1

the physiological changes in the photosynthesis/photorespiration ratio, that are characteristic of these plants, are not due to modifications of the primary reactions catalyzed by RuBP carboxylase-oxygenase. The specificity factors for the enzyme from Myriophyllum and Hydrilla bordered on the lower portion of the range of values reported for terrestrial plants, just above the values of the enzyme from green algae (Jordan and Ogren, 1981b). This placement suggests that kinetically, RuBP carboxylase-oxygenase from submersed angiosperms may have been affected by both evolutionary and environmental pressures from the potential reversion to an aquatic habitat.

The RuBP carboxylase-oxygenase protein represented a large fraction of the total soluble protein in Hydrilla, and an even larger fraction in Myriophyllum (Table 8). The difference between Myriophyllum and Hydrilla is analogous to the differences in the amount of this protein between terrestrial C_3 and C_4 plants in that, in C_4 plants, RuBP carboxylase-oxygenase protein is thought to be less abundant than in C_3 plants because of the proportionally larger amount of PEP carboxylase protein in C_4 plants (Ku et al., 1979). As a percent of the total soluble protein, the amount of RuBP carboxylase-oxygenase protein in both Myriophyllum and Hydrilla was not affected by changes in the photorespiration state (Table 8). Total soluble protein, expressed on either a chlorophyll or fresh weight basis, was less in the low photorespiration state of Myriophyllum; consequently, the amount of RuBP carboxylase-oxygenase protein was also less in the low, as compared with the high, photorespiration plants (Table 8). Chlorophyll content was less in the high photorespiration state of Hydrilla than in the low, but on a fresh weight basis, total soluble protein and, therefore, RuBP carboxylase-oxygenase protein were similar. The similar amounts of

Table 8. Chlorophyll, soluble protein and RuBP carboxylase-oxygenase (RuBPCO) protein content in Myriophyllum and Hydrilla in the low and high photorespiration (PR) states.

Plant and PR state	Chlorophyll	Soluble protein	RuBPCO	Soluble protein	RuBPCO	RuBPCO
	(mg/g fresh weight)			*(mg/mg chlorophyll)*		*(% of soluble protein)*
Myriophyllum						
Low PR	1.3	5.6	2.7	4.4	2.1	48.3
High PR	1.1	8.1	3.4	7.2	3.1	43.1
Hydrilla						
Low PR	1.2	4.1	1.4	3.6	1.2	35.1
High PR	0.3	4.1	1.5	12.7	4.7	37.4

RuBP carboxylase-oxygenase protein in the high and low photorespiration states of Hydrilla indicate that the increase in the activity of PEP carboxylase exhibited by low photorespiration state plants, if due to de novo synthesis of PEP carboxylase protein, is not sufficient to cause any substantial decrease in the observable proportion of RuBP carboxylase-oxygenase protein.

APPENDIX B
THE EFFECT OF IRRADIANCE ON THE CO_2 COMPENSATION POINTS OF *MYRIOPHYLLUM* AND *HYDRILLA*

Introduction

The irradiance level has almost no effect on the CO_2 compensation points of terrestrial C_3 and C_4 plants above the light compensation point (Chollet and Ogren, 1975). In contrast, the O_2-sensitive component of the CO_2 compensation point of C_3-C_4 intermediate species, such as *Panicum milioides* and *Moricandia arvensis*, increases considerably with decreasing light levels (Holaday et al., 1982). The effect of irradiance on the CO_2 compensation points of *Myriophyllum* and *Hydrilla* plants in the low photorespiration state was examined to determine whether, in this regard, these submersed angiosperm species, when in the low photorespiration state, are more similar to C_3 and C_4 plants or to C_3-C_4 intermediates.

Materials and Methods

The CO_2 compensation points were measured as described previously (CHAPTER II) except that the irradiance was varied using neutral density filters.

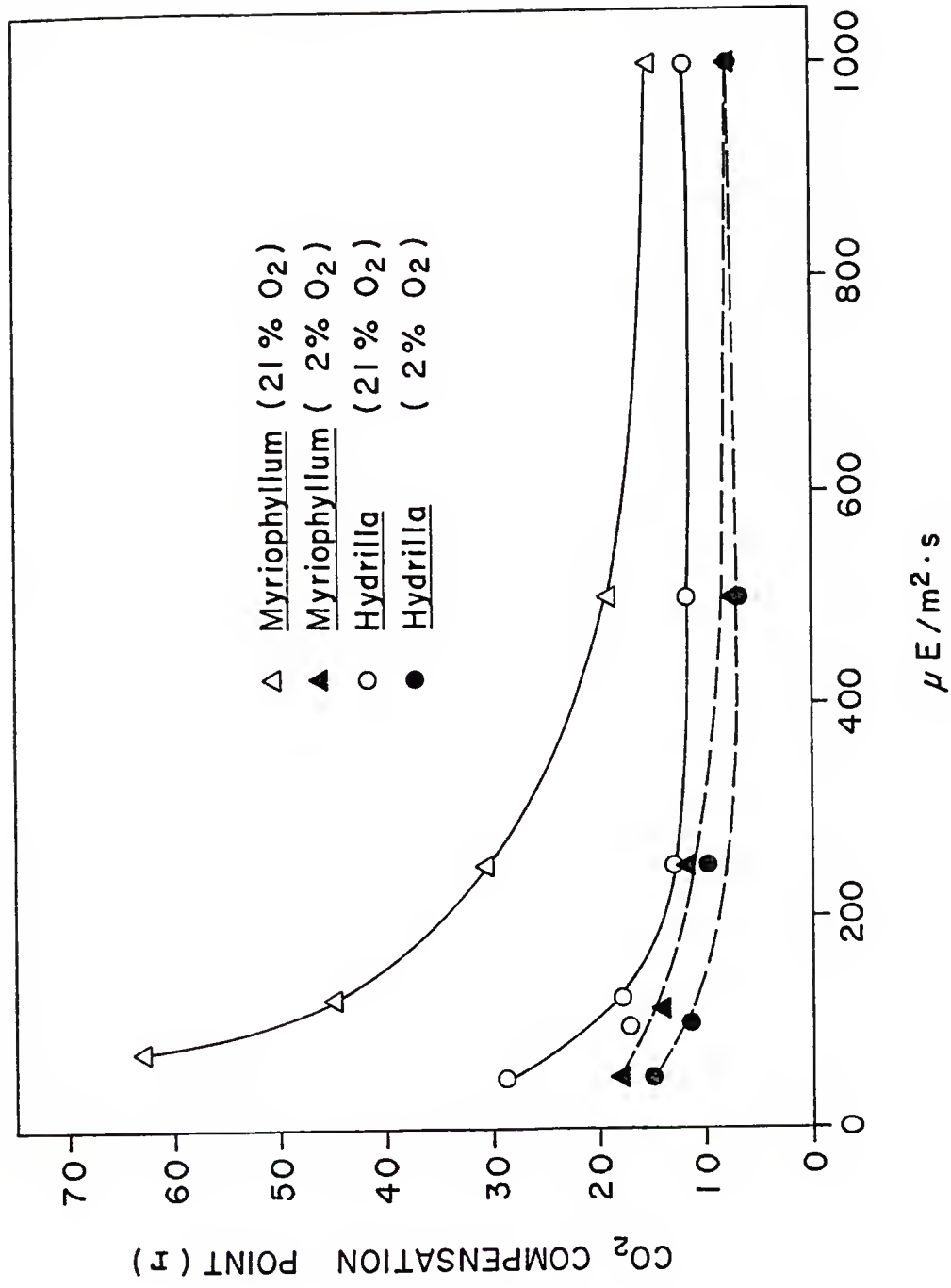
Results and Discussion

For *Myriophyllum*, the CO_2 compensation points measured at 21% O_2, but not at 1% O_2, increased considerably at irradiances less than 400

$\mu E/m^2 \cdot s$, and approached high C_3-like values below 200 $\mu E/m^2 \cdot s$ (Fig. 12). In contrast, the O_2-sensitive component of the CO_2 compensation point of low photorespiration Hydrilla exhibited only a slight increase, even at irradiances less than 100 $\mu E/m^2 \cdot s$ (Fig. 12).

As the O_2-sensitive component of the CO_2 compensation point is a reliable indicator of the extent of photorespiration in these plants (Salvucci and Bowes, 1982), the differences between Myriophyllum and Hydrilla in the response of their CO_2 compensation points to light, may be an indication that the photorespiration-reducing mechanism of Myriophyllum requires a greater energy input than the mechanism that is operating in Hydrilla.

Figure 12. The effect of irradiance on the CO_2 compensation points of *Myriophyllum* and *Hydrilla* plants in the low photorespiration (PR) states. CO_2 compensation points were measured at 30°C.


Myriophyllum (21% O2)
Myriophyllum (2% O2)
Hydrilla (21% O2)
Hydrilla (2% O2)
CO2 COMPENSATION POINT (Γ)
0
10
20
30
40
50
60
70
0
200
400
600
800
1000
μE/m2·s

APPENDIX C
DIURNAL CHANGES IN THE SENSITIVITY OF HYDRILLA PEP CARBOXYLASE TO MALATE INHIBITION

Introduction

The induction of the low photorespiration state in Hydrilla, is in several ways, reminiscent of the induction of the CAM mode in facultative CAM plants (Holaday and Bowes, 1980; Salvucci and Bowes, 1981). In the facultative CAM plant Mesembryanthemum, PEP carboxylase activity, induced in the CAM mode, has been shown to exhibit diurnal variations in the Km(PEP) and in sensitivity to malate inhibition (Winter, 1982). These variations regulate the activity of PEP carboxylase by inhibiting and/or limiting β-carboxylation during the period of the day when it would otherwise compete with RuBP carboxylase for decarboxylated CO_2 (Winter, 1982). As the potential for dual carboxylation probably exists in low photorespiration Hydrilla plants, it was of interest to know whether a similar type of regulation is exerted on PEP carboxylase in Hydrilla.

Materials and Methods

Phosphoenolpyruvate (PEP) carboxylase was assayed spectrophotometrically in 2 ml assays containing 50 mM Hepes-KOH, 2 mM $KHCO_3$, 5 mM $MgCl_2$, 5 mM DTT, 2.5 mM PEP, 0.2 mM NADH, malate, and 3 IU of malate dehydrogenase, at pH 8.0. Plant material (1 g) was ground in a Ten Broeck homogenizer at 4°C in 5 ml of 200 mM Hepes-KOH, 0.2 mM EDTA, 5 mM DTT, and 2% (w/v) PVP-40,

at pH 7.0, filtered through four layers of cheesecloth and the clarified extract was used to initiate the assay. The elapsed time from the start of the homogenization to the initiation of the assays was less than 2 min. Four simultaneous assays were performed on each extract, 2 control and 2 treatments, using the cell programmer attachment of a Cary 219 spectrophotometer.

Results and Discussion

In Table 9 are shown the effects of malate at 2 and 10 mM on PEP carboxylase activity in low photorespiration state Hydrilla plants extracted at three times of the day. Malate was most inhibitory at the end of the dark period causing 70% inhibition (Table 9). Both during the early and late stages of the light period, inhibition of PEP carboxylase by malate was about 40%; considerably lower than at 6:30 hours (Table 9). These results suggest that PEP carboxylase activity in the low photorespiration state of Hydrilla exhibits some differential sensitivity to malate inhibition over the course of the day. Unlike in CAM plants, however, during the light period, PEP carboxylase activity exhibits a relatively low degree of inhibition by malate. This observation corresponds to the high level of CO_2 incorporation into C_4 acids that can be demonstrated with Hydrilla plants during the light period (Holaday and Bowes, 1980; CHAPTER III).

Table 9. Effect of malate (2 and 10 mM) on PEP carboxylase activity rapidly extracted from low photorespiration state Hydrilla plants at three times of the day.

Time of day (hour)	Phosphoenolpyruvate Carboxylase Activity					
	Control	Malate (2 mM)	Inhibition	Control	Malate (10 mM)	Inhibition
	(μmol/mg Chl·h)		*(%)*	*(μmol/mg Chl·h)*		*(%)*
6:30	267.6	80.2	70.0	193.5	44.3	77.1
11:00	244.3	153.8	36.0	271.3	160.4	40.9
16:00	316.0	188.0	40.5	283.3	198.2	30.2

Plants were grown under a 14 h photoperiod, from 8:00 to 22:00 hours.

BIBLIOGRAPHY

Allen, E.D., Spence, D.H.N. (1981) The differential ability of aquatic plants to utilize the inorganic carbon supply in fresh waters. New Phytol. 87, 269-283

Andrews, T.J., Abel, K.M. (1979) Photosynthetic carbon metabolism in seagrasses. ^{14}C-labeling evidence for the C_3 pathway. Plant Physiol. 63, 650-656

Arnon, D.I. (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 24, 1-15

Ascencio, J.C. (1979) Phosphoenolpyruvate carboxylase kinetics and the CO_2 requirement of photosynthesis in Hydrilla verticillata. Ph.D. dissertation, University of Florida, Gainesville

Aspden, W.C., Wells, G.N. (1980) Photosynthetic carbon metabolism in seagrasses. Plant Physiol. 65, S88

Atkins, C.A., Canvin, D.T. (1971) Photosynthesis and CO_2 evolution by leaf discs: gas exchange, extraction, and ion-exchange fractionation of ^{14}C-labeled photosynthetic products. Can. J. Bot. 49, 1225-1234

Badger, M.R., Kaplan, A., Berry, J.A. (1978) A mechanism for concentrating CO_2 in Chlamydomonas reinhardtii and Anabaena variabilis and its role in photosynthetic CO_2 fixation. Carnegie Inst. Wash. Year Book 77, 251-261

Badger, M.R., Kaplan, A., Berry, J.A. (1980) Internal inorganic carbon pool of Chlamydomonas reinhardtii. Evidence for a carbon dioxide-concentrating mechanism. Plant Physiol. 66, 407-413

Bain, J.T., Proctor, M.C.F. (1980) The requirement of aquatic bryophytes for free CO_2 as an inorganic carbon source: some experimental evidence. New Phytol. 86, 393-400

Bassham, J.A., Calvin, M. (1957) The Path of Carbon in Photosynthesis. Prentice-Hall, Englewood Cliffs

Beardall, J. (1981) CO_2 accumulation by Chlorella saccharophila (Chlorophyceae) at low external pH: evidence for active transport of inorganic carbon at the chloroplast envelope. J. Phycol. 17, 371-373

Beardall, J., Raven, J.A. (1981) Transport of inorganic carbon and the 'CO_2 concentrating mechanism' in Chlorella emersonii (Chlorophyceae). J. Phycol. 17, 134-141

Beer, S., Eshel, A., Waisel, Y. (1977) Carbon metabolism in seagrasses. I. The utilization of exogenous inorganic carbon species in photosynthesis. J. Exp. Bot. 28, 1180-1189

Beer, S., Shomer-Ilan, A., Waisel, Y. (1980) Carbon metabolism in seagrasses. II. Patterns of photosynthetic CO_2 incorporation. J. Exp. Bot. 31, 1019-1026

Beer, S., Waisel, Y. (1979) Some photosynthetic carbon fixation properties of seagrasses. Aquat. Bot. 7, 129-138

Beer, S., Wetzel, R.G. (1981) Photosynthetic carbon metabolism in the submerged aquatic angiosperm Scirpus subterminalis. Plant Sci. Lett. 21, 199-207

Beer, S., Wetzel, R.G. (1982a) Photosynthetic carbon fixation pathways in Zosteria marina and three Florida seagrasses. Aquat. Bot. 13, 141-146

Beer., S., Wetzel, R.G. (1982b) Photosynthesis in submersed macrophytes of a temperate lake. Plant Physiol. 70, 488-492

Benedict, C.R., Scott, J.R. (1976) Photosynthetic carbon metabolism of a marine seagrass. Plant Physiol. 57, 876-880

Benedict, C.R., Wong, W.W.L., Wong, J.H.H. (1980) Fractionation of the stable isotopes of inorganic carbon by seagrasses. Plant Physiol. 65, 512-517

Berry, J., Boynton, J., Kaplan, A., Badger, M. (1976) Growth and photosynthesis of Chlamydomonas reinhardtii as a function of CO_2 concentration. Carnegie Inst. Wash. Year Book 76, 423-432

Best, E.P.H., Meulemans, J.T. (1979) Photosynthesis in relation to growth and dormancy in Ceratophyllum demersum. Aquat. Bot. 6, 53-65

Bird, I.F., Cornelius, M.J., Keys, A.J. (1980) Effect of carbonic anhydrase on the activity of ribulose bisphosphate carboxylase. J. Exp. Bot. 31, 365-369

Bird, I.F., Cornelius, M.J., Keys, A.J. (1982) Affinity of RuBP carboxylase for carbon dioxide and inhibition of the enzymes by oxygen. J. Exp. Bot. 33, 1004-1013

Björkman, O. (1976) Adaptive and genetic aspects of C_4 photosynthesis. In: CO_2 Metabolism and Plant Productivity, pp. 287-309, Burris, R.H., Black, C.C., eds. University Park Press, Baltimore

Black, C.C., Burris, J.E., Everson, R.G. (1976) Influence of oxygen concentration on photosynthesis in marine plants. Aust. J. Plant Physiol. 3, 81-86

Black, M.A., Maberly, S.C., Spence, D.H.N. (1981) Resistances to carbon dioxide fixation in four submerged freshwater macrophytes. New Phytol. 89, 557-569

Bowes, G., Holaday, A.S., Haller, W.T. (1979) Seasonal variation in the biomass, tuber density and photosynthetic metabolism of Hydrilla in three Florida lakes. J. Aquat. Plant Manag. 17, 61-65

Bowes, G., Holaday, A.S., Van, T.K., Haller, W.T. (1978) Photosynthetic and photorespiratory carbon metabolism in aquatic plants. In: Photosynthesis 77, Proc. Fourth Int. Congr. Photosynthesis, pp. 289-298, Hall, D.O., Coombs, J., Goodwin, T.M., eds. The Biochemical Society, London

Brown, J.M.A., Dromgoole, F.I., Towsey, M.W., Browse, J. (1974) Photosynthesis and photorespiration in aquatic macrophytes. In: Mechanisms of Regulation of Plant Growth, pp. 243-249, Bielski, R.L., Ferguson, A.R., Cresswell, M.M., eds. Royal Society of New Zealand, Wellington

Browse, J.A., Brown, J.M.A., Dromgoole, F.I. (1979a) Photosynthesis in the aquatic macrophyte Egeria densa. II. Effects of inorganic carbon conditions on ^{14}C-fixation. Aust. J. Plant Physiol. 6, 1-9

Browse, J.A., Brown, J.M.A., Dromgoole, F.I. (1980) Malate synthesis and metabolism during photosynthesis in Egeria densa Planch. Aquat. Bot. 8, 295-305

Browse, J.A., Dromgoole, F.I., Brown, J.M.A. (1979b) Photosynthesis in the aquatic macrophyte Egeria densa. III. Gas exchange studies. Aust. J. Plant Physiol. 6, 499-512

Chollet, R. (1978) Evaluation of the light/dark $^{14}CO_2$ efflux assay of photorespiration. Plant Physiol. 61, 929-932

Chollet, R., Ogren, W.L. (1975) Regulation of photorespiration in C_3 and C_4 species. Bot. Rev. 41, 137-179

Coleman, J.R., Colman, B. (1981) Inorganic carbon accumulation and photosynthesis in a blue-green alga as a function of external pH. Plant Physiol. 67, 978-982

Cooper, J.P. (1975) Photosynthesis and Productivity in Different Environments. Cambridge University Press, Cambridge

Cooper, T.G., Filner, D., Wishnick, M., Lane, M.D. (1969) The active species of "CO_2" utilized by ribulose diphosphate carboxylase. J. Biol. Chem. 244, 1081-1083

Cooper, T.G., Wood, H.G. (1971) The carboxylation of phosphoenolpyruvate and pyruvate kinase. II. The active species of "CO_2" utilized by phosphoenolpyruvate carboxylase and pyruvate kinase. J. Biol. Chem. 246, 5488-5490

De Groote, D., Kennedy, R.A. (1977) Photosynthesis in Elodea canadensis Michx. Four-carbon acid synthesis. Plant Physiol. 59, 1133-1135

Dohler, G. (1974) Carbonic anhydrase levels and enzymes of the glycolate pathway in the blue-green alga Anacystis nidulans. Planta 117, 97-99

Doohan, M.E., Newcomb, E.H. (1976) Leaf ultrastructure and $\delta^{13}C$ values of three seagrasses from the Great Barrier Reef. Aust. J. Plant Physiol. 3, 9-23

Downton, W.J.S., Bishop, D.G., Larkum, A.W.D., Osmond, C.B. (1976) Oxygen inhibition of photosynthetic oxygen evolution in marine plants. Aust. J. Plant Physiol. 3, 73-79

Ehleringer, J.R. (1979) Photosynthesis and photorespiration: biochemistry, physiology, and ecological implications. Hortscience 14, 217-222

Faquhar, G.D., O'Leary, M.H., Berry, J.A. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol. 9, 121-137

Ferrier, J.M. (1980) Apparent bicarbonate uptake and possible plasmalemma proton efflux in Chara corallina. Plant Physiol. 66, 1198-1199

Forrester, M.L., Krotkov, G., Nelson, C.D. (1966a) Effect of oxygen on photosynthesis, photorespiration and respiration on detached leaves. I. Soybean. Plant Physiol. 41, 422-427

Forrester, M.L., Krotkov, G., Nelson, C.D. (1966b) Effect of oxygen on photosynthesis, photorespiration and respiration on detached leaves. II. Corn and other monocotyledons. Plant Physiol. 41, 428-431

Frederick, S.E., Gruber, P.J., Tolbert, N.E. (1973) The occurrence of glycolate dehydrogenase and glycolate oxidase in green plants. An evolutionary survey. Plant Physiol. 52, 318-329

Gessner, F. (1955) Hydrobotanik I. Energie haushalt (Hochschulbücher für Biologie, Band 3), VEB Deutscher Verlag der Wissenschaften, Berlin

Graham, D., Atkins, C.A., Reed, M.L., Patterson, B.D., Smillie, R.M. (1971) Carbonic anhydrase, photosynthesis and light-induced pH changes. In: Photosynthesis and Photorespiration, pp. 267-274, Hatch, M.D., Osmond, C.B., Slayter, R.O., eds. Wiley-Interscience, New York

Graham, D., Smillie, R.M. (1976) Carbonate dehydratase in marine organisms of the Great Barrier Reef. Aust. J. Plant Physiol. 3, 113-119

Hanes, C.S., Isherwood, F.A. (1949) Separation of the phosphoric esters on the filter paper chromatogram. Nature 164, 1107-1112

Hatch, M.D., Boardman, N.K. (1981) The Biochemistry of Plants, Vol. 8, Academic Press, New York

Hattersley, P.W., Watson, L., Osmond, B.B. (1977) In situ immunofluorescent labeling of ribulose-1,5-bisphosphate carboxylase in leaves of C_3 and C_4 plants. Aust. J. Plant Physiol. 4, 523-539

Helder, R.J., van Harmelen, M. (1982) Carbon assimilation pattern in the submerged leaves of the aquatic angiosperm: Valisneria spiralis L. Acta Bot. Neerl. 31, 281-295

Hogetsu, D., Miyachi, S. (1979) Role of carbonic anhydrase in photosynthetic CO_2 fixation in Chlorella. Plant Cell Physiol. 20, 747-756

Holaday, A.S., Bowes, G. (1980) C_4 acid metabolism and dark CO_2 fixation in a submersed aquatic macrophyte (Hydrilla verticillata). Plant Physiol. 65, 331-335

Holaday, A.S., Harrison, A.T., Chollet, R. (1982) Photosynthetic/photorespiratory CO_2 exchange characteristics of the C_3-C_4 intermediate species, Moricandia arvensis. Plant Sci. Lett. 27, 181-189

Holaday, A.S., Salvucci, M.E., Bowes,G. (in press) Variable photosynthesis/photorespiration ratios in Hydrilla and other submersed aquatic macrophyte species. Can. J. Bot., in press

Holtum, J.A.M., Winter, K. (1982) Activity of enzymes of carbon metabolism during the induction of Crassulacean acid metabolism in Mesembryanthemum crystallinum L. Planta 155, 8-16

Hough, R.A. (1974) Photorespiration and productivity in submersed aquatic vascular plants. Limnol. Oceanogr. 19, 912-927

Hough, R.A. (1976) Light and dark respiration and release of organic carbon in marine macrophytes of the Great Barrier Reef. Aust. J. Plant Physiol. 3, 63-68

Hough, R.A., Wetzel, R.G. (1972) A ^{14}C-assay for photorespiration in aquatic plants. Plant Physiol. 49, 987-990

Hough, R.A., Wetzel, R.G. (1977) Photosynthetic pathways of some aquatic plants. Aquat. Bot. 3, 297-313

Hough, R.A., Wetzel, R.G. (1978) Photorespiration and CO_2 compensation point in Najas flexilis. Limnol. Oceanogr. 23, 719-724

Hutchinson, G.E. (1975) A Treatise on Limnology, Vol. 3, Limnological Botany. John Wiley and Sons, New York

Jarvis, P.G. (1971) The estimation of resistances to carbon dioxide transfer. In: Plant Photosynthetic Production; Manual of Methods, pp. 49-110, Sestak, Z., Catsky, J., Jarvis, P.G., eds. W. Junk, The Hague

Jordan, D.B., Ogren, W.L. (1981a) A sensitive assay procedure for simultaneous determination of ribulose-1,5-bisphosphate carboxylase and oxygenase activities. Plant Physiol. 67, 237-245

Jordan, D.B., Ogren, W.L. (1981b) Species variation in the specificity of ribulose bisphosphate carboxylase-oxygenase. Nature 291, 513-515

Kaplan, A., Zenvirth, D., Reinhold, L., Berry, J.A. (1982) Involvement of a primary electrogenic pump in the mechanism for HCO_3^- uptake by the cyanobacterium Anabaena variabilis. Plant Physiol. 69, 978-982

Keeley, J. (1981) Isoetes howelii: a submerged aquatic CAM plant? Am. J. Bot. 68, 420-424

Keeley, J., Bowes, G. (1982) Gas exchange characteristics of the submerged aquatic Crassulacean acid metabolism plant, Isoetes howelli. Plant Physiol. 70, 1455-1458

Kluge, M., Ting, I.P. (1978) Crassulacean Acid Metabolism. Analysis of an Ecological Adaptation. Springer-Verlag, New York

Ku, M.S.B., Schmitt, M.R., Edwards, G.E. (1979) Quantitative determination of RuBP carboxylase-oxygenase protein in leaves of several C_3 and C_4 plants. J. Exp. Bot. 30, 89-98

Kumarasinghe, K.S., Keys, A.J., Whittingham, C.P. (1977) Effects of certain inhibitors on photorespiration by wheat segments. J. Exp. Bot. 28, 1163-1168

Kung, S.D., Chollet, R., Marsho, T.V. (1980) Crystallization and assay procedures of tobacco ribulose-1,5-bisphosphate carboxylase-oxygenase. In: Methods of Enzymology, Vol. 69, pp. 326-335, San Pietro, A., ed. Academic Press, New York

Laing, W.A., Ogren, W.L., Hageman, R.H. (1974) Regulation of soybean net photosynthetic CO_2 fixation by the interaction of CO_2, O_2 and ribulose-1,5-diphosphate carboxylase. Plant Physiol. 54, 678-685

Laurell, C.-B. (1972) Electroimmuno assay. Scan. J. Clin. Lab. Invest. 29, suppl. 124, 21-37

Lloyd, N.D.H., Canvin, D.T. (1977) Photosynthesis and photorespiration in sunflower selections. Can. J. Bot. 55, 3006-3012

Lloyd, N.D.H., Canvin, D.T., Bristow, J.M. (1977) Photosynthesis and photorespiration in submersed aquatic vascular plants. Can. J. Bot. 55, 3001-3005

Lorimer, G.H. (1981) The carboxylation and oxygenation of ribulose-1, 5-bisphosphate: the primary events of photosynthesis and photorespiration. Ann. Rev. Plant Physiol. 32, 349-383

Lucas, W.J. (1980) Control and synchronization of HCO_3^- and OH^- transport during photosynthetic assimilation of exogenous HCO_3^-. In: Plant Membrane Transport: Current Conceptual Issues, pp. 317-327, Spanswick, R.M., Lucas, W.J., Dainty, J., eds. Elseveir/North Holland Biomedical Press, Amsterdam

Lucas, W.J., Ferrier, J.M., Dainty, J. (1977) Plasmalemma transport of OH^- in Chara corallina: dynamics of activation and deactivation. J. Membr. Biol. 32, 49-73

Ludwig, L.J., Canvin, D.T. (1971) An open gas-exchange system for the simultaneous measurement of the CO_2 and $^{14}CO_2$ fluxes from leaves. Can. J. Bot. 49, 1299-1313

Lüttge, U., Higinbotham, N. (1979) Transport in Plants. Springer-Verlag, New York

Maren, T.H. (1967) Carbonic anhydrase: chemistry, physiology and inhibition. Physiol. Rev. 47, 595-781

McCracken, M.D., Adams, M.S., Titus, J., Stone, W. (1975) Diurnal course of photosynthesis in Myriophyllum spicatum and Oedogonium. Oikos 26, 355-361

Morgan, J.A., Brown, R.H. (1979) Photosynthesis in grass species differing in carbon dioxide fixation pathways. II. A search for species with intermediate gas exchange and anatomical characteristics. Plant Physiol. 64, 257-262

O'Leary, M.H. (1981) Carbon isotope fractionation in plants. Phytochem. 20, 553-568

Osmond, C.B. (1981) Photorespiration and photoinhibition: some implications for the energetics of photosynthesis. Biochim. Biophys. Acta 639,77-98

Osmond, C.G., Holtum, J.A.M. (1981) Crassulacean acid metabolism. In: The Biochemistry of Plants, Vol. 8, pp. 283-327, Hatch, M.D., Boardman, N.K., eds. Academic Press, New York

Osmond, C.B., Valanne, N., Haslam, S.M., Votila, P., Roksondic, Z. (1981) Comparison of $\delta^{13}C$ values in leaves of aquatic macrophytes from different habitats in Britain and Finland; some implications for photosynthetic processes in aquatic plants. Oecologia 50, 117-124

Perrot-Rechenmann, C., Vidal, J., Brulfert, J., Burlet, A., Gadal, P. (1982) A comparative immunocytochemical localization study of phosphoenolpyruvate carboxylase in leaves of higher plants. Planta 155, 24-30

Pratt, S.G., Rand, L. (1979) Thin-layer chromatographic separation of ^{14}C-labeled metabolites from photosynthate. J. of Liquid Chromatog. 2, 239-253

Prins, H.B.A., O'Brien, J., Zanstra, P.E. (1982a) Bicarbonate utilization in aquatic angiosperms. pH and CO_2 concentration at the leaf surface. In: Studies on Aquatic Vascular Plants, pp. 112-119, Symoens, J.J., Hooper, S.S., Compére, P., eds. Royal Society of Belgium, Brussels

Prins, H.B.A., Snel, J.F.H., Zanstra, P.E., Helder, R.J. (1982b) The mechanism of bicarbonate assimilation by the polar leaves of Potamogeton and Elodea. Plant, Cell and Environ. 5, 207-214

Rathnam, C.K.M., Chollet, R. (1979) Photosynthetic carbon metabolism in Panicum milioides, a C_3-C_4 intermediate species: evidence for a limited C_4 dicarboxylic acid pathway of photosynthesis. Biochim. Biophys. Acta 548, 500-519

Rathnam, C.K.M., Chollet, R. (1980) Photosynthetic carbon metabolism in C_4 plants and C_3-C_4 intermediate species. Prog. Phytochem. 6, 1-48

Raven, J.A. (1970) Exogenous inorganic carbon sources in plant photosynthesis. Biol. Rev. 45, 167-221

Raven, J., Beardall, J., Griffiths, H. (1982) Inorganic C-sources for Lemanea, Cladophora, and Ranunculus in a fast-flowing stream: measurements of gas exchange and of carbon isotope ratio and their ecological implications. Oecologia 53, 68-78

Rejda, J.M., Johal, S., Chollet, R. (1981) Enzymatic and physiochemical characteristics of ribulose-1,5-bisphosphate carboxylase/oxygenase from diploid and tetraploid cultivars of perennial ryegrass. Arch. Biochem. Biophys. 210, 617-624

Salvucci, M.E., Bowes, G. (1981) Induction of reduced photorespiratory activity in submersed and amphibious aquatic macrophytes. Plant Physiol. 67, 335-340

Salvucci, M.E., Bowes, G. (1982) Photosynthetic and photorespiratory responses of the aerial and submerged leaves of Myriophyllum brasiliense. Aquat. Bot. 13, 147-164

Sculthorpe, C.D. (1971) The Biology of Aquatic Vascular Plants. E. Arnold, London

Servaites, J.C., Ogren, W.L. (1977) Chemical inhibition of the glycolate pathway in soybean leaf cells. Plant Physiol. 60, 461-466

Shelp, B.J., Canvin, D.T. (1980) Photorespiration and oxygen inhibition of photosynthesis in Chlorella pyrenoidosa. Plant Physiol. 65, 780-784

Shelp, B.J., Canvin, D.T. (1981) Photorespiration in air and high CO_2-grown Chlorella pyrenoidosa. Plant Physiol. 68, 1500-1503

Smith, F.A., Walker, N.A. (1980) Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^- and to carbon isotope discrimination. New Phytol. 86, 245-259

Somerville, C.R., Ogren, W.L. (1982) Genetic modification of photorespiration. Trends in Biochem. Sci. 7, 171-174

Søndergaard, M. (1979) Light and dark respiration and the effect of the lacunal system on refixation of CO_2 in submersed aquatic plants. Aquat. Bot. 6, 269-283

Søndergaard, M., Wetzel, R.G. (1981) Photorespiration and internal recycling of CO_2 in the submersed angiosperm Scirpus subterminalis. Can. J. Bot. 58, 591-597

Spaulding, M.H., Spreitzer, R.J., Ogren, W.L. (1982) High CO_2 requiring mutants of Chlamydomonas. Plant Physiol. 69, S102

Spector, T. (1978) Refinement of the coomassie blue method of protein quantitation. A simple and linear spectrophotometric assay for 0.5 to 50 µg of protein. Anal. Biochem. 86, 142-146

Stanley, R.A., Naylor, A.W. (1972) Photosynthesis in Eurasian watermilfoil (Myriophyllum spicatum L.). Plant Physiol. 50, 149-151

Stanley, R.A., Naylor, A.W. (1973) Glycolate metabolism in Eruasian watermilfoil (Myriophyllum spicatum L.). Physiol. Plant. 29, 60-63

Steeman Neilson, E. (1947) Photosynthesis of aquatic plants with special reference to the carbon-sources. Dansk. Bot. Arkiv. 8, 3-71

Tichá, I., Cátsky, J. (1981) Photosynthetic characteristics during the ontogenesis of leaves. 5. Carbon dioxide compensation concentration. Photosynthetica 15, 401-428

Tolbert, N.E. (1976) Glycolate oxidase and glycolate dehydrogenase in marine algae and plants. Aust. J. Plant Physiol. 3, 129-132

Tolbert, N.E. (1981) Metabolic pathways in peroxisomes and glyoxisomes. Ann. Rev. Biochem. 50, 133-157

Touchstone, J.C., Dobbins, M.F. (1978) Practice of Thin-layer Chromatography. John Wiley and Sons, New York

Tsuzuki, M., Miyachi, S. (1979) Effects of CO_2 concentration during growth and of ethoxyzolamide on CO_2 compensation point of Chlorella. FEBS Lett. 103, 221-223

Tsuzuki, M., Shiraiwa, Y., Miyachi, S. (1980) Role of carbonic anhydrase in Chlorella derived from kinetic analysis of $^{14}CO_2$ fixation. Plant Cell Physiol. 21, 677-688

Valanne, N., Aro, E.-M., Rintamaki, E. (1982) Leaf and chloroplast structure of two Ranunculus species. Aquat. Bot. 12, 13-22

Van, T.K., Haller, W.T., Bowes, G. (1976) Comparison of the photosynthetic characteristics of three submersed aquatic plants. Plant Physiol. 58, 761-768

Van, T.K., Haller, W.T., Bowes, G. (1978) Some aspects of the competitive biology of Hydrilla. Proc. Europ. Weed Res. Soc., Fifth Sympos. on Aquat. Weeds, pp. 117-126

Walker, N.A. (1980) The transport systems of charophyte and chlorophyte giant algae and their integration into modes of behaviour. In: Plant Membrane Transport: Current Conceptual Issues, pp. 287-300, Spanswick, R.M., Lucas, W.J., Dainty, J., eds. Elseveir/North Holland Biomedical Press, Amsterdam

Walker, N.A., Smith, F.A., Cathers, I.R. (1980) Bicarbonate assimilation by freshwater charophytes and higher plants: I. Membrane transport of bicarbonate ions is not proven. J. Memb. Biol. 57, 51-58

Weaver, C.I., Wetzel, R.G. (1980) Carbonic anhydrase levels and internal lacunar CO_2 concentrations in aquatic macrophytes. Aquat. Bot. 8, 173-186

Westlake, D.F. (1975) Primary production of freshwater macrophytes. In: Photosynthesis and Productivity in Different Environments, pp. 189-206, Cooper, J.P., ed. Cambridge University Press, Cambridge

Wilkinson, G.N. (1961) Statistical estimations in enzyme kinetics. Biochem. J. 80, 324-332

Williams, S.L., McRoy, C.P. (1982) Seagrass productivity: the effect of light on carbon uptake. Aquat. Bot. 12, 321-344

Winter, K. (1978) Short-term fixation of ^{14}carbon by the submerged angiosperm Potamogeton pectinatus. J. Exp. Bot. 29, 1169-1172

Winter, K. (1982) Regulation of PEP carboxylase in CAM plants. In: Crassulacean Acid Metabolism, Proc. Fifth Ann. Sympos. Bot., pp. 153-169, Ting, I.P., Gibbs, M., eds. American Soc. Plant Physiol., Rockville

Yeoh, H.-H., Badger, M.R., Watson, L. (1981) Variations in kinetic properties of ribulose-1,5-bisphosphate carboxylases among plants. Plant Physiol. 67, 1151-1155

Zelitch, I. (1968) Investigation on photorespiration with a sensitive ^{14}C-assay. Plant Physiol. 43, 1829-1837

BIOGRAPHICAL SKETCH

Michael Edward Salvucci was born in Drexel Hill, Pennsylvania, on June 18, 1956. He received his primary and secondary school education in Drexel Hill, graduating from Monsignor Bonner High School in June 1974.

He entered the Pennsylvania State University at University Park, Pennsylvania, in September of 1974 and graduated with a Bachelor of Science degree in biology in March of 1978. On August 19, 1978, he married Cynthia S. Spaulding in Versailles, Kentucky. In September of that same year, he entered the University of Florida to begin graduate study within the Department of Botany in the area of photosynthesis. After graduating with a Master of Science degree in botany in June 1978, he continued his graduate study as a doctoral candidate, working on a Ph.D. degree in botany with a minor in biochemistry. Upon completion of the degree requirements, he will assume a postdoctoral research associate position with the Photosynthesis Group at the University of Illinois.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

George Bowes

George Bowes, Chairman
Associate Professor of Botany

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Thomas E. Humphreys

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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